JOURNAL OF AGRICULTURAL RESEARCH

VOL. 35 WASHINGTON, D. C., OCTOBER 1, 1927 NO. 7

A CYTOLOGICAL STUDY OF CERATOSTOMELLA ADIPOSUM (BUTL.) COMB. NOV., THE BLACK-ROT FUNGUS OF SUGAR CANE [1]

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INTRODUCTION

The fungus which is the subject of this study was first described by Butler [3] as *Sphaeronema adiposum*. It is common in the sugar-cane fields of India, and does considerable damage to seed pieces under certain conditions, which Butler fully describes in his article. Normally, however, it is a weak parasite of little or no economic importance.

Perhaps on account of the unusual behavior of the asci and owing to their small size, the true nature of the fungus was overlooked and consequently was not given its proper taxonomic position. Thus, besides establishing the true nature of the fungus, the phenomena observed are of interest with reference to the development of the Ascomycetes in general, and particularly of the Sphaeriales, which have been studied but little cytologically.

The literature dealing with the nuclear phenomena of the Ascomycetes is extensive, covering many different types of various degrees of sexuality. This literature has been thoroughly reviewed by Gwynne-Vaughan.[4] In this paper it will be mentioned only such observations of others as bear directly on those in this study. The cytological work here reported was undertaken as essential in confirming the decision that the so-called pycnidial stage of the "seed-cane" black-rot fungus was in reality a perfect or sexual stage.

THE HABIT OF THE FUNGUS

The fungus was found in Terrebonne Parish, La., during the winter of 1924, when it caused slight damage to seed cane stored in sawdust. This damage was confined mostly to the cut ends. In the spring of 1926, however, the fungus caused great loss in a field of cane at Ellendale plantation near Houma, La.

Two varieties of sugar cane, P. O. J. 213 and P. O. J. 36, were received from Cairo, Ga., and planted at Ellendale plantation dur-

[1] Received for publication Apr. 18, 1927; issued November, 1927.

[2] The writer is under obligation to Mana K. Sartoris for making the drawings illustrating this paper.

[3] BUTLER, E. J. FUNGUS DISEASES OF SUGAR CANE IN BENGAL. India Dept. Agr. Mem., Bot. Ser., v. 1, no. 3, 53 p., illus. 1906.

[4] GWYNNE-VAUGHAN, H. C. I. FRASER. FUNGI: ASCOMYCETES, USTILAGINALES, UREDINALES. 232 p., illus. Cambridge [Eng.], 1922.

ing March, 1926. About two weeks after planting, a large proportion of the seed pieces became infected by the black-rot fungus. In cases where the soil was not well packed around the seed pieces, the fungus fruited. The long-beaked perithecia were produced in abundance on the cut ends of the cane (fig. 1, A and B), and at times the entire cane was covered by a cottony mass of black hyphae (fig. 1, G and H), which bore over its entire surface the large spiny endoconidia (fig. 1, C to F). The mycelium did not seem to invade the interior of the host but turned the tissue a dark purple color. In advanced stages the cane was nearly black throughout, showed a soft, watery texture, and emitted an odor similar to that produced by *Thielaviopsis ethacetica*.

Ideal conditions for the development of the fungus were found to be frequent rains, with intermittent periods of warm weather, and loosely packed, lumpy soil, which allowed large air pockets around the seed pieces.

In November, just prior to the harvesting of the cane, the amount of damage was estimated as follows: In the case of the variety P. O. J. 36 the stand was reduced to about 20 per cent of normal, and in the case of the variety P. O. J. 213 to about 30 per cent of normal. The pieces that escaped the attack of the fungus produced normal appearing stools.

CYTOLOGY

METHODS OF PROCEDURE

The fungus was obtained in pure culture by sowing single endoconidia on nutrient agar in Petri dishes, and by transferring a group of ascospores from the tip of the beak of the perithecium. (Fig. 1, A.) The ascospores, being embedded in a white, fatlike substance which clings to the tip of the beak in a little translucent globule, were easily transferred to Petri plates. Since the globule was held from 3 to 5 mm. above the surface of the plate, most of the cultures were uncontaminated. The perfect stage was produced by a culture that arose from a single endoconidium. Single ascospore cultures were not made. Butler,[5] however, made single-spore cultures from his Sphaeronema and found that a culture from one supposed pycnospore produced all of the stages. Cultures were made in Petri dishes, and the material for this study was taken from time to time until all of the stages were obtained. The material was killed in Flemming's solution, embedded in paraffin, sectioned, and stained in the usual manner. Sections were cut from 4μ to 7μ thick and were stained in Haidenhain's haematoxylin.

ASEXUAL DEVELOPMENT

The asexual stage of the fungus has been adequately described by Butler.[5] The vegetative mycelium is mostly uninucleate. When the mycelium is growing rapidly, however, cells may be multinucleate, a condition arising in many cases through fragmentation. The endoconidia, which are binucleate, are extremely variable in size and shape, ranging from elongated hyaline spores resembling the micro-

[5] BUTLER, E. J. Op. cit.

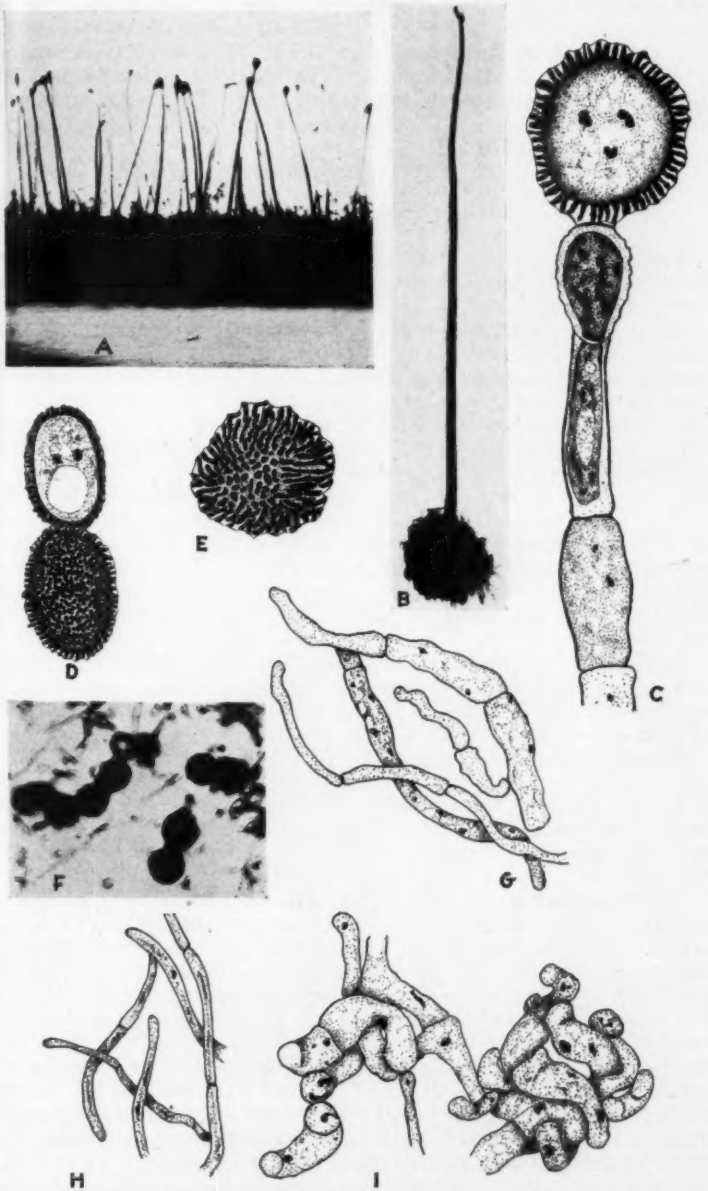

FIG. 1.—A, Habit of black-rot fungus, showing the long beaks with globules of spores at the top, × 4.5; B, a mature perithecium, × 21; C, conidiophore with a mature endoconidium and two others in early stages of formation, × 750; D, the oblong hyaline endoconidia, × 750; E, surface drawing of a mature globose endoconidium, × 750; F, photomicrograph of chains of mature endoconidia, × 260; G and H, vegetative (feeding) hyphae, × 750; I, coils of hyphae which are the perithecium initials, × 750.

conidia of *Thielaviopsis ethacetica* to large rounded ones of a deep brown color. (Fig. 1, C to F.) The exospores of the mature conidia are roughened or spiny and very thick. The same conidiophores produce the two kinds of conidia. The hyaline ones are formed first, and later the large, round, deep-brown ones. These endoconidia are formed endogenously (fig. 1, C), and measure 4.5μ to 18μ by 9μ to 25μ.

SEXUAL DEVELOPMENT

DEVELOPMENT OF THE PERITHECIUM

The perithecium is initiated by a coil of hyphae (fig. 1, I), in which there appears to be no fusion of nuclei of adjacent cells or of adjacent hyphae, although the coil is formed by somewhat enlarged hyphae from different sources, i. e., not by a single hypha. Coils similar to those described by Elliott [6] for *Ceratostomella fimbriata* were seen, but there was no differentiation of hyphae that could be designated as antheridia and oogonia. The coil becomes rather large and is composed of several layers before the fertile hyphae, which produce the asci, are differentiated. When the coil or perithecium is about 80 microns in cross section, a hypha in the center of the coil stains a deep blue with Haidenhain's haematoxylin. (Fig. 2, A.) This marks the beginning of the ascogenous hyphae. Also at the same time or shortly thereafter a meristematic group of hyphae, which are instrumental in the development of the beak, arise just above the fertile hyphae. (Fig. 2, D.) This area is always directly above the point of attachment of the perithecium to the substratum.

At this time the perithecium is spherical and is covered with radiating long dark-brown hyphae, which give it a Chaetomiumlike appearance. As it increases in size a cavity is formed at the center (fig. 2, C and D), which is lined by irregular swollen ascogenous hyphae and filled with large thin-walled cells (fig. 2, C to F), which are used up as the fertile hyphae develop. It seems quite evident that these are food-storage cells. In the early stages the ascogenous hyphae are coenocytic. The nuclei are dividing rapidly, in many cases by means of fragmentation. They seem to be in pairs, but this condition may be due to rapid growth. (Fig. 2, C.)

The branching ascogenous hyphae grow across the cavity, between the large thin-walled sterile cells and around the inner wall of the cavity. Whenever the fertile hyphae come in contact with the large sterile cells or with the thick-walled cells of the true wall of the perithecium, they become permanently attached and are more or less independent of the original base. When the point of development at which the asci are being formed is reached, the ascogenous hyphae appear to be rising from all parts of the inner wall of the perithecium. (Fig. 3, A, B, and C.) Asci near the base of the beak are matured first. (Fig. 3, A.) The development continues downward and outward until the sides of the perithecium are reached and the ascogenous hyphae and nutrient cells are exhausted. (Fig. 3, C.)

[6] ELLIOTT, J. A. A CYTOLOGICAL STUDY OF CERATOSTOMELLA FIMBRIATA (E. & H.) ELLIOTT. Phytopathology 15: 417–422, illus. 1925.

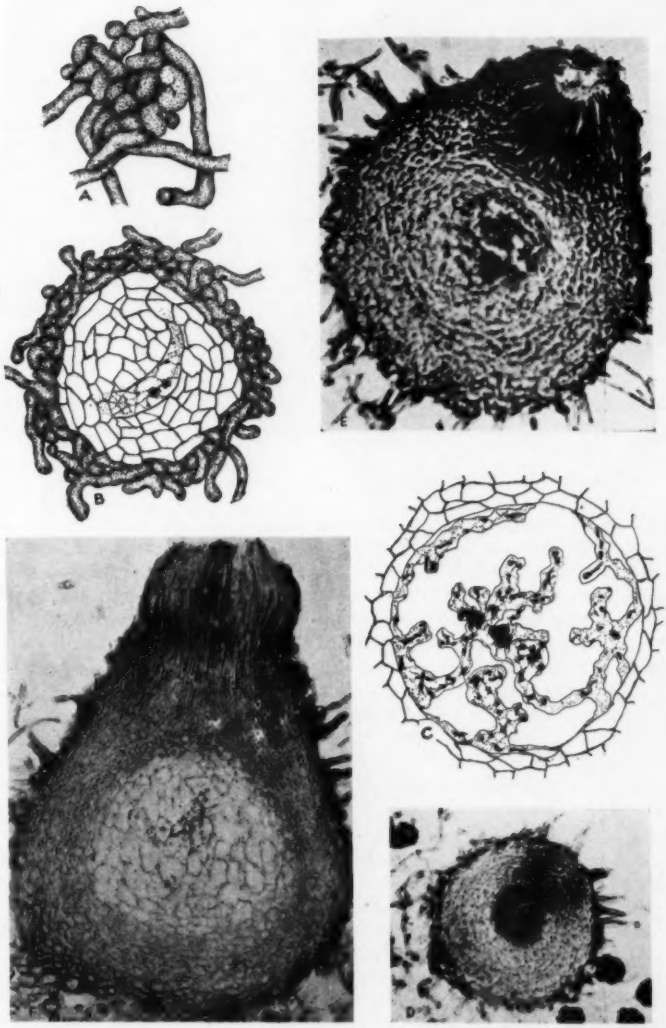

FIG. 2.—A, A coil of hyphae in a more advanced stage than the coils shown in Figure 1, I, × 590; B, a young perithecium, showing the differentiation of the first ascogenous hyphae, × 590; C, a part of the cavity of a young perithecium with branching coenocytic ascogenous hyphae and the thin-walled cells of the cavity, × 590; D, a young perithecium, showing the ascogenous hyphae in the central cavity and above them the meristematic group of hyphae which develop into the beak, × 177; E and F, young perithecia, showing early stages in the development of the beak, × 177

Development of the Ascus

The ascogenous hyphae are coenocytic and remain so until the ascus mother cells are about to be developed. Then cross walls are laid down, beginning at the terminal end of each ascogenous hypha, forming binucleate ascus mother cells. (Fig. 3, D and E.) During the growth of the ascus mother cell the two nuclei fuse to form a single fusion nucleus. (Fig. 3, E and F.) This union is followed almost immediately by a series of three divisions, producing eight nuclei. (Fig. 3, G, H, and I.) Then in the usual fashion cell walls are laid down around each nucleus (fig. 3, J and K), cutting out eight irregular spores which later become spherical and then crescent shaped (fig. 3, L). At this time the spores are biseriate and the ascus is cylindrical to pyriform and measures 10μ by 20μ to 12μ by 25μ. Either while the spores are in a spherical form or later the ascus wall begins to degenerate. In the meantime a translucent vacuolate fatty substance is formed within the ascus which persists even after the wall degenerates, holding the ascospores in groups. (Fig. 3, C.) The ascospores may continue their development for some time after the dissolution of the ascus wall. When mature they measure 3.5μ by 6.5μ to 4μ by 8μ and are crescent shaped. (Fig. 3, C and M.)

Development of the Beak

The development of the beak in the Sphaeriales has not been studied. Elliott[7] studied the life history of *Ceratostomella fimbriata* but did not describe the formation of the beak. As mentioned above, simultaneously with the differentiation of the ascogenous hyphae a meristematic area is formed at the apex of the perithecium. (Fig. 2, D.) The hyphae that form this tissue arise from opposite points of the inner wall of the perithecium. They first grow toward each other, until there is left only a small passage between them. They then turn upward, the hyphae running parallel to each other. (Fig. 2, E and F.) At the same time thick-walled hyphae from the true wall of the perithecium grow parallel to the inner thin-walled hyphae, enveloping them and extending beyond them. (Fig. 3, A, and fig. 4, A and B.) The whole forms a compact, fasciculated, rigid structure. As the beak grows the central canal begins to develop at the base. The central thin-walled hyphae are dissolved (fig. 4, C), so that longitudinal sections and cross sections show a central canal surrounded by layers of thick-walled hyphae (fig. 4, D, and F). When the beak has attained its full height of 2 to 6 mm., that portion of the outer wall extending beyond the growing points spreads out in the form of a fringe. The beak is always developed at right angles to the substratum, since it is not influenced by gravity or light.

Meanwhile the mature spores and the fatty vacuolated substance in which they are embedded completely fill the cavity of the perithecium. This develops sufficient hydrostatic pressure to force some ascospores in a portion of the fatty medium up the canal to the mouth of the beak. Here they gather in a translucent droplet, held in place by the fimbriate appendages. (Fig. 4, E.) The re-

[7] Elliott, J. A. Op. cit.

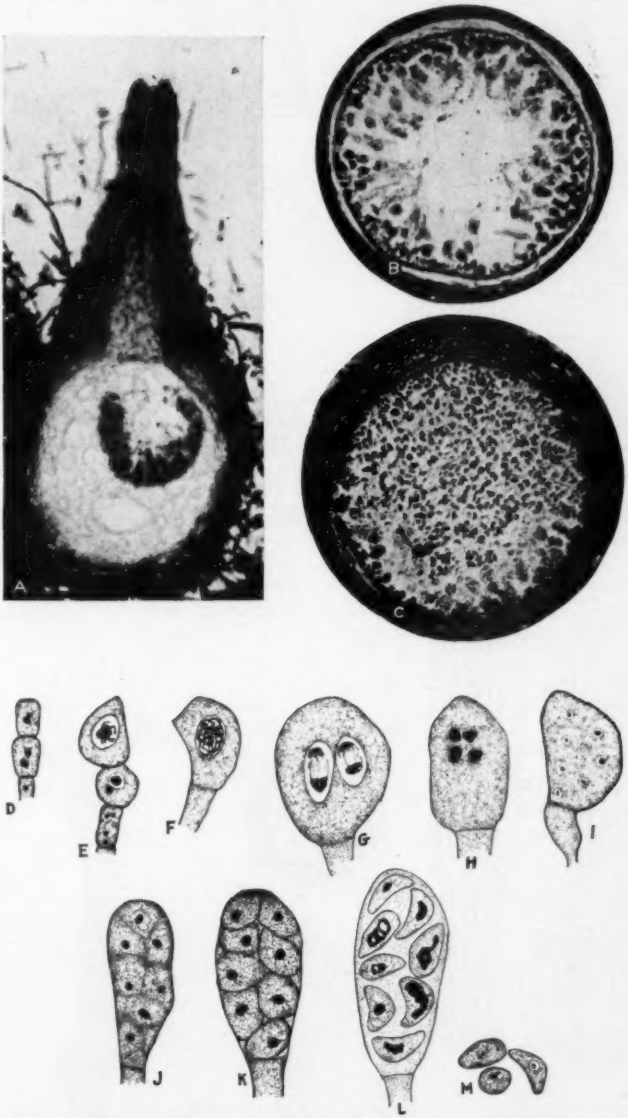

FIG. 3.—A, A later stage in the development of the beak, which shows also the ascogenous hyphae, × 165; B, cross section of a perithecium, showing stages in the maturation of the asci, × 165; C, cross section of a mature perithecium, showing the cavity filled with ascospores and the fatty medium, × 165; D, the binucleate ascus mother cell formed at the end of an ascogenous hypha, × 1100; E, stages of nuclear fusion, × 1100; F, the fusion nucleus, × 1100; G, the first division of the nucleus, × 1100; H, the second division of the nucleus, × 1100; I, the third division of the nucleus, × 1100; J, cleavages in the cytoplasm around the nuclei, × 1100; K, an ascus with eight rounded ascospores, × 1100; L, a mature ascus with eight crescent-shaped spores, × 1100; M, mature crescent-shaped ascospores, × 1100

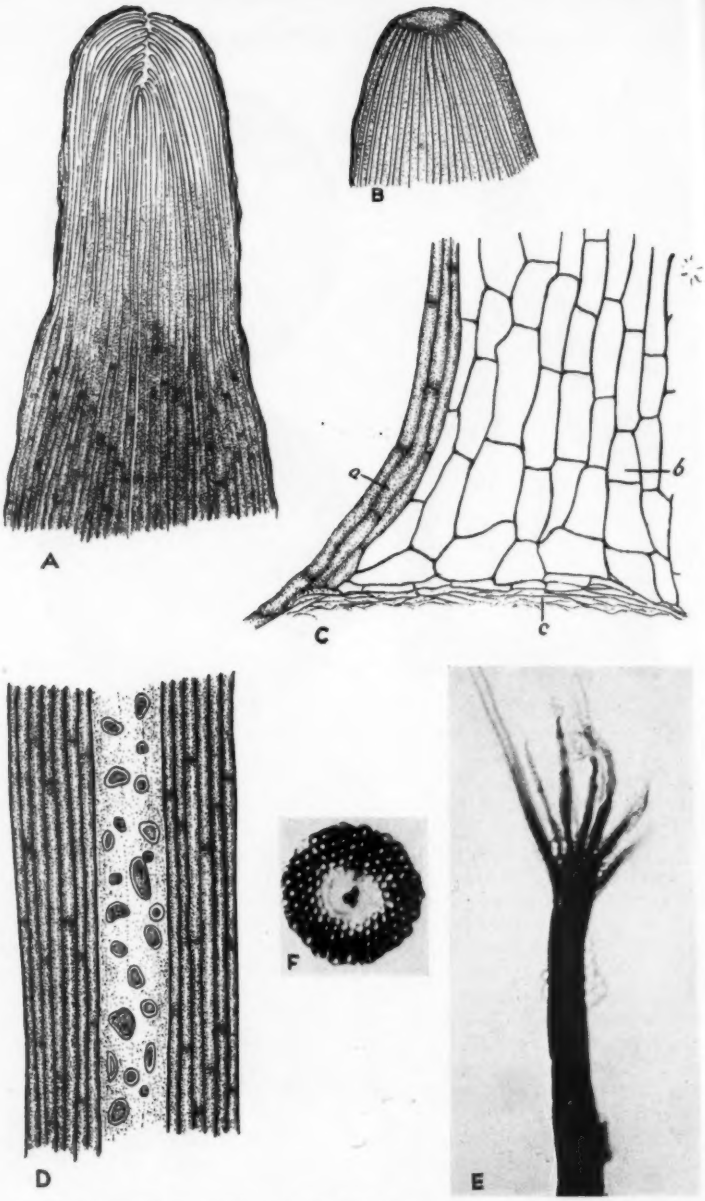

FIG. 4.—A and B, The tip of the beak, showing the growing point enveloped by the hyphae of the true wall of the perithecium, × 770. C, A longitudinal section of the beak showing (*a*) thick-walled cells forming the true wall of the perithecium; (*b*) large thin-walled cells that are dissolved away, thus forming the central canal; and (*c*) degenerating hyphae at the base of the beak and lining the cavity of the perithecium, × 1150. D, A longitudinal section of a mature beak, showing the thick wall and the central canal with ascospores being forced out, × 770. E, The appendages around the mouth of the beak, × 150. F, A cross section of the beak, showing the structure of the wall, the central canal, and spores in the canal

mainder of the ascospores are not disseminated until the perithecium degenerates.

When the droplet is first formed it is white and translucent, but with age it turns yellow and finally brown. This change in color is probably due to the action of oxidases. If the droplet is not disturbed, the original shape is retained with very little loss in size for a long time. The ascospores remain viable for about three months. The fatty substance seems to aid in accomplishing three results—first, to carry the spores to the mouth of the beak; second, to aid in the dissemination of the spores by sticking to insects that may crawl over the culture; and third, to keep the spores from drying out.

SUMMARY

The results of this study show that the fungus described by Butler as *Sphaeronema adiposum* is in reality an ascomycete belonging to the genus Ceratostomella. Its name therefore becomes *Ceratostomella adiposum* (Butler) Sartoris, comb. nov.

Under certain conditions the fungus causes great loss to seed cane, but normally it is a weak parasite.

The perithecium is initiated by a coil of enlarged hyphae, but differentiation of the ascogenous hyphae does not take place until the young perithecium is quite large. The ascogenous hyphae are coenocytic during most of their development, but just prior to ascus formation cross walls are laid down, forming binucleate ascus mother cells. These nuclei fuse to produce a fusion nucleus, which by three divisions forms eight nuclei which become ascospore nuclei. The beak is formed by a meristematic group of hyphae which are differentiated at the apex of the young perithecium. The ascospores are forced up the canal of the long beak by the development of hydrostatic pressure in the base of the perithecium.

SOIL FACTORS INFLUENCING THE DEVELOPMENT OF THE MOSAIC DISEASE IN WINTER WHEAT[1]

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INTRODUCTION

Early studies by McKinney (*8*)[3] indicated that environmental factors during the seedling stage are important in the development of the rosetted condition of the mosaic disease of winter wheat. Beginning with a normal seeding date, he found that the percentages of rosetted plants from periodic seedings were less in the late sowings than in the early ones. He found further that the disease practically was controlled where emergence did not occur until the following spring.

The causal agent of the wheat mosaic disease, referred to in previous publications (*8, 10*), is soil borne, and the disease has its natural origin or point of attack on the underground parts of plants in the seedling stage. In fact, until the recently successful inoculation experiments by McKinney (*10*) the only method of obtaining the disease was by sowing susceptible varieties in infested soil during the natural growing season for winter wheat. Even now this is the only method whereby relatively high percentages of diseased plants can be obtained. The very important soil relation of this disease certainly possesses especial significance and furnishes an opportunity for study along lines which thus far have been either impossible or difficult for other virus diseases of the mosaic type.

What are the possible rôles which environmental factors are playing in this disease? Are the two distinct varietal responses, namely, the rosette and the mosaic-mottling phases, influenced by the same environmental factors? If so, are they affected in the same way and to the same degree? It was in an attempt to answer these questions that this investigation was started. A study of the environmental conditions with particular reference to the soil phase was essential to a proper understanding of the disease and of its occurrence and distribution in general and in restricted areas. Accordingly, experiments have been conducted on the influences of constant and fluctuating soil temperatures and soil moistures during the critical stages for infection. The influences of the stage of seedling development and of the time factor in relation to soil temperature have been followed. Further, the effects of surface sterilization of the underground parts of infected seedlings with bichloride of mercury have been determined. Although these studies are rather preliminary, it is believed that the results are of sufficient importance to warrant their publication at this time.

[1] Received for publication June 3, 1927; issued November, 1927. This paper reports results obtained in cooperative investigations between the Agricultural Experiment Station of the University of Wisconsin and the Bureau of Plant Industry, U. S. Department of Agriculture.

[2] The writer is grateful to H. H. McKinney for assistance in certain phases of the experiments and for suggestions concerning the manuscript; also to C. R. Ball and A. G. Johnson for editorial suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 613.

EXPERIMENTS

The studies reported in this paper were conducted in experimental plots out of doors at Madison, Wis., during the winter-wheat seasons of 1923–24 and 1924–25. All of the experiments reported have been conducted in season; that is, the grain was sown during the fall and the plants passed through their usual period of winter dormancy. Infection occurs in the seedling stage during the autumnal period, and consequently the most attention has been focused on this stage and on this period.

Two very susceptible varieties of winter wheat, namely, Harvest Queen and Currell, have been tested simultaneously in all experiments except one. The Harvest Queen variety is an excellent one for expressing both the rosette and the mosaic-mottling phases, and the Currell variety is an excellent one for expressing only the mosaic mottling. These various symptoms have been described fully in the previous papers (*8*, *10*, *11*, *12*). All of the available evidence seems to indicate that the rosette and the mosaic-mottling phases are different expressions of the same disease.

The naturally infested soil used in these experiments was transported to Madison from the American Bottoms of the Mississippi River near Granite City, Ill., across the river from St. Louis, Mo. This soil is a fine sedimentary type and is spoken of as a heavy gumbo. The very consistent and uniform results obtained in all the experiments indicate that this soil was more or less uniformly infested. Fertile loam soil obtained in the vicinity of Madison, Wis., served as a control in the experiments.

The air and soil temperatures were accurately recorded by thermographs which were periodically checked. The soil-temperature data represent the conditions at a depth of 2 inches. Daily fluctuating temperatures have been converted by taking a mean of the maximum and minimum daily temperatures and by expressing these in terms of a mean weekly value. Any method of evaluating fluctuating temperatures is open to criticism, but the method employed here was thoroughly adequate for the purpose of the experiments. Only the soil-temperature data for the seedling stage have been included in this paper. The air-temperature data were very similar to the soil-temperature data, with the exception, of course, of greater daily fluctuations.

TANK-TRANSPLANTING EXPERIMENT, 1924–25

Methods

The studies of controlled soil temperature and moisture were combined and conducted jointly. The Wisconsin soil-temperature tanks, as described by Jones (*4*, *5*) and as modified by Dickson (*1*), McKinney (*9*), and others, were employed. In order that the seedlings might experience the usual fall conditions out of doors and thereby develop sufficient hardiness to withstand the severity of the winter, these experiments were not conducted in the greenhouse. Four of the tanks were arranged side by side in the outdoor experimental plot, and were securely walled with boards, covered with tar roofing, and further insulated with sawdust between the tanks and the walls. The 30° and 23° C. tanks were electrically heated and thermostatically controlled; the 16° tank was regulated by the

adjustment of the inflow of water from the city water main; and the 10° tank was supplied with cracked ice several times a day, depending upon weather conditions. All regulations were made on the basis of the soil temperature 1½ inches beneath the surface and in the center of the cans. Considering the outdoor conditions, the soil temperatures were surprisingly constant. The tanks intended to be held at 30°, 23°, and 16°, respectively, were regulated to within an average of 0.5° to 1.0° of the desired temperature. Somewhat greater variations occurred in the tank at the lowest temperature, in which the temperature ranged from 8° to 12°, although there was a fairly constant 10° value during most of the period. The extremes of this temperature range occurred during relatively short periods when the ice was applied or when the sun shone intensely. The spreads between the temperatures of the various tanks, however, were sufficiently wide to offset the influence of such minor variations and to enable the experiments to serve their purpose.

The infested soil employed in each series of tank experiments was thoroughly pulverized, screened, mixed, and adjusted to the desired moisture content as expressed in percentage of its moisture-holding capacity. The moisture-holding capacity of this soil was 60 per cent. A weighed quantity of soil was placed in each container, and the soil moisture was kept as nearly constant as possible during the course of the experiments by daily weighing the cultures and bringing them to constant weight with water of the same temperature as the tank. Tap water was used in all the adjustments, and it was applied at the surface. Despite the variations in moisture distributions and the crude method used in attempting to regulate it, the adjusted soils certainly represent low, midheight, and high moisture contents. Granite City soil adjusted to a soil-moisture content of 42 to 45 per cent of its moisture-holding capacity possesses ideal friability for tillage and plant growth; that at 52 to 58 per cent of capacity represents the highest moisture content at which the soil can be satisfactorily worked; whereas soil at 30 to 32 per cent of capacity is extremely dry, and soaked seeds require considerable periods for germination in it.

The fertile loam soil used for checks possessed a moisture-holding capacity of 30 per cent. Checks were employed at only one soil-moisture content, namely, 42 to 45 per cent of the moisture-holding capacity.

Containers of two sizes were employed. In the first experiment, ordinary No. 2 pea cans were used; in the second, metal containers 6 inches in diameter and 10 inches in depth were used. A distinct advantage is offered by the small containers in that they permit of easy removal and manipulation of plants with the least possible injury. On the other hand, the increased number of small containers greatly increases the routine daily weighing and watering, and this is a decided disadvantage. In general, however, the No. 2 pea cans are well adapted to seeding, transplanting, and overwintering studies such as are reported in this paper.

The grain to be sown was hand picked and soaked overnight in water at room temperature. This was done in order to facilitate and hasten germination, especially in the soil adjusted to the lowest moisture content, thus giving the seedlings the longest and most uniform period possible under the controlled conditions. In each

of the smaller containers 12 kernels were evenly distributed, and five such containers constituted a group. In each of the larger containers 60 kernels were similarly sown, and only one container was used for each combination. Thus, a total of 60 kernels was tested for each variety in each moisture content at each temperature. In all cases the grain was sown at a uniform depth of 1.5 inches.

At night, and during the day before showers occurred, the tanks were covered with a waterproof canvas which protected the plants against all moisture other than that which was applied to maintain a constant weight. The canvas was spread over a ridgepole fully 4 feet above the plants, and the sides of the canvas were securely attached to the wall surrounding the tanks. The ends of the canvas were left open to permit normal air circulation.

Sowings in the first experiment were made September 23, 1924, and in the second experiment November 1, 1924. Thirty days from date of seeding the plants were carefully removed, the soil was washed away, the plants were allowed to soak in several changes of tap water, all soil particles were removed by means of agitation in water with a camel's-hair brush, and the plants were further rinsed in running tap water and transplanted to metal containers filled with disease-free soil. The plants showing severe Helminthosporium infection on the underground parts were segregated and transplanted separately. Holes were cut in the bottoms of the cans for drainage, and slots were cut in the side from the top to the soil line to permit surface drainage. The cans then were buried in the ground to the soil line, mulched with hay, and allowed to overwinter in this condition.

Effects on Host Development

The two varieties of winter wheat tested proved very sensitive to different soil temperatures, which indicated a different type of metabolism for the seedlings growing under each different set of environmental conditions described. The results obtained are shown in Table 1, and they agree well with those published by Dickson (*1*), McKinney (*9*), and others.

In general, the germination of both the Harvest Queen and Currell varieties was hastened by high soil temperature and high soil moisture. Germination was somewhat poor and erratic at both the midheight and high-moisture contents in a temperature of 30° C. and at the low moisture contents in temperatures of 10° and 16° C. No germination whatever occurred at the low moisture contents in temperatures of 23° and 30° C., despite the fact that the seeds were soaked overnight in water at room temperature before sowing. The seedlings emerged promptly and uniformly, except those in the soil of low moisture content at temperatures of 10° and 16° C. Prolonged periods were required for the emergence of these seedlings. At the time of transplanting, only about one-third to two-thirds of the germinated kernels in these cases had produced emerging seedlings.

The type and degree of root development were distinctly different at the high and at the low temperatures. No very marked variations, other than that of extent of growth, occurred with the different moistures at any particular temperature. At the two lower temperatures, 10° and 16° C., the root systems were well developed, very

extensive, long, white, fleshy, smooth, and nonbranching. At the two higher temperatures, 23° and 30° C., the root systems were poorly developed, short, brown, fibrous, rough, and branching. The type of soil appeared to influence the length of root development to some extent, the average root system being 1 to several inches longer in the control sandy-loam soil than in the infested gumbo soil.

TABLE 1.—*Influence of constant soil temperatures and constant soil moistures on the development of the mosaic disease of Harvest Queen and Currell winter wheats in naturally infested soil*

Variety and soil temperature	Soil moisture	Germination	Emergence	Time elapsed from seeding to emergence	Seedlings transplanted Oct. 23, 1924						Mosaic disease in 1925			
					Leaf stage of development	Length of—			Helminthosporium infection		Number of plants surviving	Mottling		Rosette, June 4
						Tops	Roots	Subcoronal internode	Mild [a]	Severe [b]		April 28	May 12	
Harvest Queen:	*P.ct*	*P. ct.*	*P. ct.*	*Ds.*		*Ins.*	*Ins.*	*Inches*	*P.ct*	*P.ct*		*P. ct.*	*P. ct.*	*P. ct.*
10° C	30	83.3	32.0	[c]30	1st				0	0	50	0	0	0
	42	100	100	11	2d	4.5	3.5	0	0	0	60	0	[d]1.7	[e]1.7
	52	100	100	9	3d	5	4.5	0	0	0	60	[f]100	[f]100	[g]37.7
16° C	30	[h]86.7	66.7	[c]19	1st	4.3	3.5	[i]1	15.0	0	40	0	0	0
	42	100	100	7	3d	6.5	4	[j]0.25 to 1	15	13.3	60	0	13	13
	52	98.3	100	5	3d	7	5	[j].25 to 1	13.6	3.4	59	[f]100	[f]100	[k]58.5
23° C	30	0												
	42	91.6	100	7	2d to 3d	8	3.5	[j].25 to 1	32.7	16.4	55	0	0	0
	52	100	100	3	4th	9	5.5	[i]1 to 1.5	0	56.7	60	0	0	0
30° C	30	0												
	42	53.3	100	7	1st to 3d	7.5	3	[i]1	31.3	3.1	32	0	0	0
	52	81.7	100	4	4th	9	5	1 to 1.5	18.4	36.7	49	0	0	0
Currell:														
10° C	30	70	45.2	[c]30	1st to 2d		3–4		0	0	42	0	0	
	42	100	100	11	3d		4	0	0	0	60	0	[d]5	
	52	98.3	98.3	10	3d	5.8	4	0	0	0	59	0	[l]50	
16° C	30	46.7	46.7	[c]19	1st to 2d	4.5	3	0	14.3	0	28	0	0	
	42	98.3	98.3	7	3d	6.5	4	[j].25 to .75	10.2	0	59	[l]17.7	[l]6.3	
	52	85	85	5	3d	7	5	[j].25 to .75	17.6	0	51	[l]18.5	[f]50	
23° C	30	0												
	42	65	65	7	3d to 4th	8.5	3.5	[j].25 to 1	0	51.3	39	0	0	
	52	91.6	91.6	3	4th	9	4	[j].50 to 1	0	73.3	55	0	0	
30° C	30	0												
	42	25	25	8	2d to 3d	8.3	3	[j].125 to 1	0	13.3	15	0	0	
	52	41.7	41.7	4	4th	9.5	5	[j]1 to 1.5	0	68	25	0	0	

[a] One to several faint lesions on coleoptile, outer sheaths, subcrown, and roots.
[b] Coleoptile dark brown or black and numerous lesions on main tissue, outer sheaths, subcrown or roots.
[c] Very irregular emergence.
[d] Faint mottling.
[e] Intermediate type.
[f] Conspicuous mottling.
[g] Typical rosette, 30.2 per cent; intermediate, 7.5 per cent.
[h] Twelve recently germinated and weakly plants not transplanted.
[i] General.
[j] Variable.
[k] Typical rosette, 47.2 per cent; intermediate, 11.3 per cent.
[l] Midcolor mottling.

The subcoronal internode responded readily to the different environmental conditions. On the whole, the structures were more elongated in the Harvest Queen variety than in the Currell. With the high soil-moisture content, at the two high temperatures, 23°

and 30° C., the elongation was greatest, practically all the plants showing such internodes measuring 1 to 1.5 inches in length. With the median soil-moisture content at the same two temperatures, on the other hand, these structures generally were shorter and considerably more variable in length. Elongation was reduced still further and appeared more variable at 16° C., whereas at 10° C. it was practically inhibited. Soil temperature and soil moisture, therefore, are important factors influencing the development of the subcoronal internode, but the large variations frequently occurring in seedlings at a particular temperature-moisture combination indicate that other factors also operate.

The development of the coleoptile is just the opposite of that of the subcoronal internode. High temperatures of 23° to 30° C. inhibited rapid elongation of the coleoptile, the growing point of the culm generally rupturing the coleoptile before emerging from the soil. At 16° and 10° C., on the other hand, the elongation of the coleoptile proceeded at a much faster rate than that of the culm, and the subsequent rupture of the coleoptile did not occur until some time after its emergence from the soil.

The largest production of tops, expressed in terms of average length from the first node to the tip of the longest leaves, occurred at 23° and 30° C., followed in turn by that at 16° and 10° C. Furthermore, the length varied more or less directly with the percentage of moisture present in the soil. No tillers had developed in either variety at the time the plants were removed from the tanks, namely, 30 days after date of seeding.

The Harvest Queen variety withstood the severity of the winter at Madison, Wis., much better than the Currell variety. In the second tank series, when the seedlings experienced relatively low air temperatures during several nights prior to their removal, interesting correlations were noted between low-temperature injury and the conditions under which the seedlings were growing. Previous high soil temperature and high soil moisture predisposed the seedlings to severe winter injury. For example, at 30° C. the Currell seedlings in soil with the high and median moisture contents were killed, whereas those in soil of low moisture content showed slight top injury, but a weakening of the base at the soil line. Similar though less severe relations were noted at 23° C. At 16° C. the seedlings in the soil of high moisture content were generally injured and weakened at the bases, but no injury occurred at the other two soil-moisture contents. No injury whatever was noted in any of the seedlings grown at a temperature of 10° C.

In general, stronger, healthier, stockier, more vigorous, and darker green plants were obtained at the two low temperatures, 10° and 16° C., than at the two higher temperatures, 23° and 30° C. The plants grown at the higher temperatures were rather spindling, somewhat lighter green in color, and possessed a poorly developed root system as compared to the others. In addition, they were quite generally and heavily infected with Helminthosporium.

Effects on Disease Development

Results obtained from the Harvest Queen variety of wheat are shown in Table 1 and in Figures 1 and 2. It is very evident that the disease developed only in plants which passed their early seedling

stage within restricted soil-temperature and soil-moisture ranges. At the two high soil temperatures, 23° and 30° C., neither rosette nor mottling nor any indications of them appeared, whereas at the two low soil temperatures, 16° and 10° C., rosette and mottling occurred. A high soil-moisture content favored the disease, whereas its occurrence was less marked at a medium soil-moisture content. No indications of either rosette or mottling occurred at the low soil-moisture content, namely, 30 per cent. The soil-moisture relations appeared very definite and important. The percentage of plants manifesting the rosetted condition is higher and the type of rosette is more extreme at 16° than at 10° C.

Comparing the results in the high soil-moisture content at the two favorable soil temperatures, it will be seen that 37.7 per cent of the plants grown at 10° C., and 58.5 per cent of those grown at 16° C., showed the rosetted condition. In both cases approximately three-

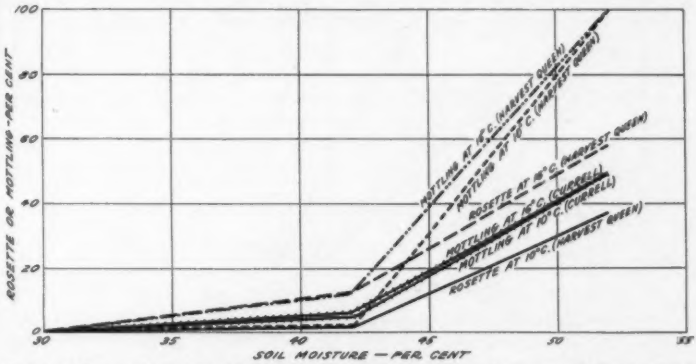

FIG. 1.—Influence of constant soil temperatures and constant soil moistures on the development of the mosaic disease of winter wheat, as expressed in terms of rosette and mosaic mottling by the Harvest Queen variety, and of mosaic mottling by the Currell variety, when grown in naturally infested soil for a period of 30 days from date of seeding, 1924. (Same data plotted differently in fig. 2)

fourths of the plants represented in these percentages manifested distinct rosette symptoms, the remaining one-fourth representing somewhat less extreme types. Under medium soil-moisture content, at each of the two favorable low temperatures, there was a marked reduction in rosetted plants, the percentage having decreased to 1.7 and 13 per cent, respectively.

Up to this point only the rosette phase of the disease has been considered. It is evident from a further examination of the data in Table 1 that the soil-temperature range for mosaic mottling is the same as that for the rosette condition. Whereas the percentages of rosetted plants in the soil of highest moisture content were higher at a temperature of 16° than at 10° C., the percentages of mosaic-mottled plants under similar conditions were the same at both temperatures, 100 per cent. Thus, the mottling phase is more general than the rosetted phase. Furthermore, the mottling in both instances was abundant at different observations extending over a period of several weeks. With a medium soil-moisture content, at the two favorable low soil temperatures, the percentages of mottled plants were greatly reduced, only the rosetted plants showing mot-

tling. The soil-moisture relations for mottling, therefore, resemble those for rosette.

Helminthosporium infection was severe in the infested soil under both the medium and high moisture contents at temperatures of 23° and 30° C. Under these conditions the coleoptiles were entirely brown or black and the subterranean parts showed numerous severe lesions. No indications of the mosaic disease either in the form of rosette or of mottling appeared on such plants. Furthermore, the plants at the soil temperature of 16° C. which showed comparatively mild Helminthosporium infection had neither more nor better rosette or mottling symptoms than those which were free from Helminthosporium. Comparing the soil-temperature range and the soil-

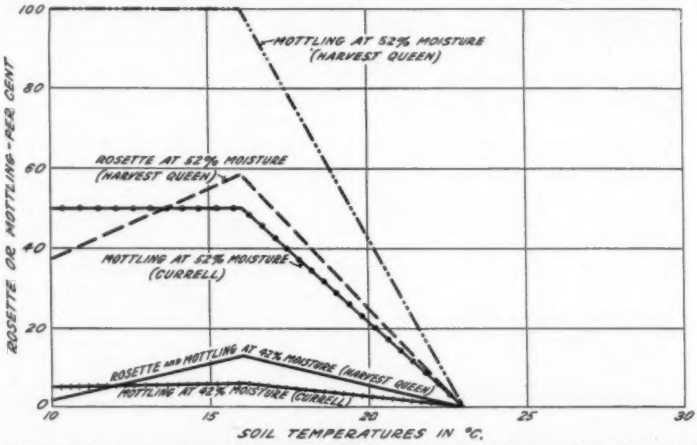

FIG. 2.—Influence of constant soil temperatures and constant soil moistures on the development of the mosaic disease of winter wheat, as expressed in terms of rosette and mosaic mottling by the Harvest Queen variety, and of mosaic mottling by the Currell variety, when grown in naturally infested soil for a period of 30 days from date of seeding. (Same data plotted differently in fig. 1)

temperature optima for the Helminthosporium disease of wheat, as obtained by McKinney (*9*), with those for the mosaic disease, as obtained in these present experiments, it is evident that the temperature relations of the two diseases are very different. This is further conclusive evidence that Helminthosporium is not the cause of the mosaic disease, as McKinney, Webb, and Dungan (*12*) previously have shown that it is not the cause of rosette.

The results with the Currell variety are assembled in Table 1 and the curves plotted to illustrate these results appear in Figures 1and 2. Soil temperatures and soil moistures which were favorable to both the rosette and mottling phases of mosaic disease in the Harvest Queen variety also are favorable to the mottling of the Currell variety. No mottling occurred at 23° and 30° C. The curves representing mottling in Currell in the corresponding soil moistures, at temperatures of 10° and 16° C., are practically identical and are very similar to the curves for rosette at a temperature of 16° C. The number of mottled plants in each case is 6 to 8 per cent less than the number of rosetted plants. Unlike the rosette phase in Harvest Queen, which

occurred in higher percentages at 16° than at 10° C., the mottling in Currell occurred in approximately equal quantities at the two soil temperatures. The two different soil temperatures, however, did influence the intensity of the mottling, even if they did not appreciably alter the percentages. For example, in the medium and high soil-moisture contents, respectively, the mottling was faint to midmotley at a temperature of 10°, as compared with midmotley to conspicuous at a temperature of 16° C.

Soil moisture proved to be equally as important a factor in the mottling of the Currell variety as in the rosette and mottling of the Harvest Queen variety. At the favorable temperatures no mottling whatever was evidenced with the low soil-moisture content, despite the fact that the plants were well into the first-leaf stage of development at the time of removal from the infested soil. Only 5 to 6 per cent of the plants showed a faint to midcolor mottling at the medium soil-moisture content, whereas approximately 50 per cent showed a midcolor to conspicuous mottling at the high soil-moisture content. The effects of the soil-moisture relations were very striking.

The Harvest Queen and Currell plants developed normally in the check soil, and the plants at each soil temperature were very similar to the corresponding plants in the infested soil adjusted to medium or high moisture contents. In no case was there the slightest indication of rosette or mottling of the plants which served as controls.

FIELD EXPERIMENT ON DATE OF SEEDING, 1924-25

Eight successive seedings of Harvest Queen and Currell winter wheats were made in infested soil, at two-week intervals during the fall of 1924, beginning August 4. The sowings were made in flats measuring 12 by 14 by 8 inches, and the infested soil composed the upper 4-inch layer in the flat. Disease-free Madison loam made up the lower 3-inch layer in the flat. This method consistently has given results similar to those obtained where the infested soil filled the entire depth of the flat. Fifty hand-picked kernels of the particular variety were sown in each flat, 25 kernels in each of two rows, at a depth of 1.5 inches. The soil was watered frequently, depending upon the degree of dryness. The flats were sunk in the ground at a slight angle and two holes were bored directly above the soil line in the lower end of each flat. This insured elimination of surface water before freezing was possible, and this precaution, and the use of a hay mulch, minimized subsequent winter injury to the plants.

Effects on Host Development

The data concerning this experiment are given in Table 2. The percentage of germination and the number of days before emergence were variables. In general, however, the best germination occurred in the early seedings and poorest germination occurred in the late seedings. Similarly, the number of days required for emergence was smallest in the two earliest seedings and somewhat greater in the later seedings.

The Currell plants of the seventh seeding date (October 27) were severely injured by air temperatures below freezing November 7, 8, and 9, and they were killed during the winter. Injury was not as

general and severe in the corresponding seeding of the Harvest Queen variety, some of the plants of which survived the winter. The final sowings were made November 10, but both varieties were completely killed soon after emergence. The plants of the other periodic sowings passed into the dormant period in various stages. Those of the two August seedings produced a very heavy, rank, and luxuriant top growth, while those of the last seeding were in the youngest seedling stage. The different intervening seedings produced plants which were of various stages between these two extremes. The aerial parts of the plants seeded earliest—August 4 and 18—were heavily infected with rust.

TABLE 2.—*Influence of daily fluctuating soil temperatures on the development of the mosaic disease of winter wheat, as expressed by Harvest Queen and Currell varieties sown on different dates in naturally infested soil in 1924*

Date of sowing	Period	Average weekly soil temperatures (° C.)			Germination	Time elapsed between seeding and emergence	Record of disease development in 1925			
							Harvest Queen		Currell	
		Mean	Maximum	Minimum			Rosette, June 16	Mottling, May 1	Mottling, May 1	Mottling, May 13
					P. ct.	*Days*	*P. ct.*			
Aug. 4	Aug. 4–10	22.9	26.4	19.5	90	4	0	(a)	Midmotley.	Midmotley.
	Aug. 11–17	23.1	28.7	17.4						
Aug. 18	Aug. 18–24	21.3	25.0	17.6	86	4	2.3	(a)	do.	Do.
	Aug. 25–31	21.8	25.7	17.8						
Sept. 1	Sept. 1–7	15.8	18.8	12.9	56	7	82.1	Midmotley.	do.	Do.
	Sept. 8–14	12.7	14.6	10.7						
Sept. 15	Sept. 15–21	14.8	18.0	11.5	46	5	78.3	do.	do.	Do.
	Sept. 22–28	13.3	16.3	10.4						
Sept. 29	Sept. 29–Oct. 5	10.7	12.8	8.5	72	6	75.0	do.	Conspicuous.[b]	Do.
	Oct. 6–12	10.3	11.7	8.9						
Oct. 13	Oct. 13–19	10.1	11.5	8.7	48	8	79.1	do.	do.[b]	Do.
	Oct. 20–26	5.4	6.5	4.3						
Oct. 27	Oct. 27–Nov. 2	6.1	7.8	4.4	38	7	47.4	do.	(c)	(c).
	Nov. 3–9	3.1	4.7	1.6						
Nov. 10	Nov. 10–16	2.0	2.9	1.1	(d)	(d)	(d)	(d)	(d)	(d).
	Nov. 17–23	.1	.9	.7						

[a] Mottling so faint as to be questionable.
[b] Mottling very general.
[c] Plants killed during winter.
[d] Plants killed while emerging.

EFFECTS ON DISEASE DEVELOPMENT

The mottling data for both the Harvest Queen and Currell varieties and the rosette data for the Harvest Queen variety appear in Table 2. Figure 3 contains the curve representing the percentages of rosetted plants occurring in the different sowings as related to the mean weekly soil temperature. The disease symptoms were not as severe as noted in previous years, because of the cool and dry spring in which the plants developed. Many diseased plants showed a tendency to send up apparently normal tillers or to recover almost entirely in some instances.

In the case of Harvest Queen, it is interesting to note that no plants of the August 4 seeding showed the rosetted condition, and only a relatively few, 2.3 per cent, of the August 18 seeding showed it. These field results agree excellently with those obtained in the experiments under controlled soil temperature and moisture. The percentages of plants showing rosette in the next four dates of seeding, September 1, 15, and 29 and October 13, respectively, were uniformly high, ranging from 75 to 82 per cent. In these sowings, the mean weekly soil temperature ranged from 16° C. in early September to 10° C. in the middle of October. The rosette symptoms in plants from the September 15 and 29 seeding dates were very pronounced, and proliferation at the crown was excessive. Prolif-

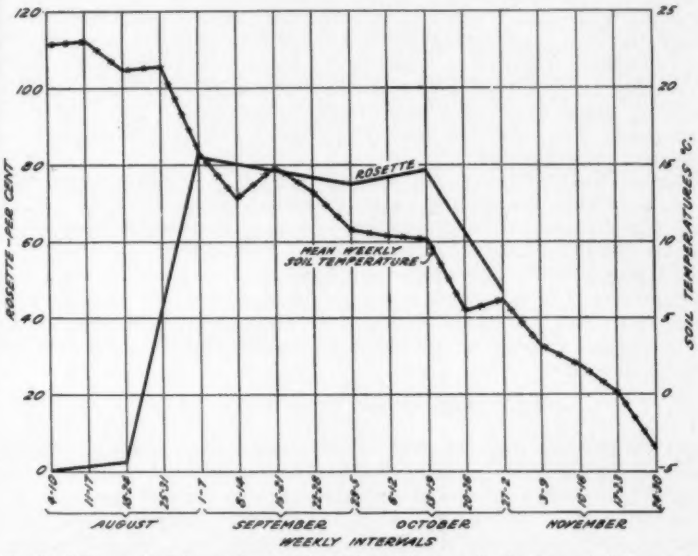

FIG. 3.—Influence of soil temperatures, under field conditions, on the development of the mosaic disease of winter wheat, as expressed in terms of rosette in the Harvest Queen wheat sown on different dates in naturally infested soil, in 1924

eration in a few plants of the October 13 seeding equaled that in the previously mentioned plants, but, in general, it was less. From the last seeding (October 27) in which the seedlings withstood the winter, the rosetted plants totaled 47.4 per cent, which was a considerably lower value than that furnished by the four earlier sowings. The symptoms also were much less marked.

A very faint, questionable, and not general mottling occurred in the plants from both the August 4 and August 18 sowings. The mottling in the seedings of September 1, 15, and 29 and October 13 and 27 was midmotley to conspicuous and occurred generally. Thus in the two earliest seedings, in which the young seedlings experienced a mean weekly soil temperature of 21° C. and above, little or no rosette developed, and the mottling was very faint. The relatively low temperature for a few hours each night probably has an active

influence and may account for the faint mottling and the mere trace of rosette. These results agree well with those obtained in the tank experiments in which the soil temperature was held constant.

The Currell variety showed different degrees and percentages of mottling in the several sowings. The mottling was faint to midmotley and not general in the two August sowings, midmotley to conspicuous and general in the next two, and conspicuous and general in the last two. These observations were made May 1, and those made May 13 showed that the intensity of the mottling was about equal in each of the different sowings. Thus it seems that mottling in the Currell variety can develop during the seedling stage at slightly higher mean soil temperatures than can either mottling or rosette in the Harvest Queen variety. These relations are slightly at variance with those shown in the constant soil-temperature studies, where the soil-temperature and soil-moisture range was the same for both mottling and rosette. In this connection, however, later results show that infection necessary for mottling may take place at later seedling stages, in shorter time intervals, and over a wider temperature range, than that for rosette. This would tend to explain the apparent discrepancies.

While the results of the different periodic sowings have been considered thus far on the basis of soil temperature, it is realized that the duration of exposure to infection, as related to environmental conditions, is an important one. However, the data from this experiment do not throw any light on this particular angle of the problem. In the transplanting experiments which are described next, the effect of varied duration of exposure to infection at different periods during the fall has been studied and the data obtained are significant.

FIELD TRANSPLANTING EXPERIMENTS, 1923–24

On October 3, 1923, Harvest Queen wheat was sown in infested soil and in steam-sterilized Madison soil used as a control. One hundred No. 2 pea cans filled with the infested soil and 200 such cans filled with the control soil were employed. Seven kernels were sown in each container, and periodic watering kept the soil in good condition.

Transplantings were made at regular weekly intervals during the fall and winter, but more frequently during the earliest stages of seedling development. Each transplanting involved 60 seedlings, namely, 20 seedlings from noninfested soil to infested soil, 20 seedlings from infested soil to noninfested soil, and 20 seedlings from noninfested soil to noninfested soil, to serve as controls.

The seedlings were removed and transplanted with the greatest care in all cases. The entire contents of the cans were removed, the mass was washed carefully until the roots of the plants were clean, the roots of each seedling were washed repeatedly, and all particles of soil were removed with a camel's hair brush; and the underground parts of the seedlings were sterilized for five minutes in 1:1,000 $HgCl_2$ and finally washed repeatedly in running tap water. The seedlings were transplanted to the proper soil in similar containers, five seedlings being placed in each.

Immediately after the original seeding and after the different transplantings, the containers were buried in the ground to their

soil line, and slots were cut from the upper edges of the cans to the soil line to permit drainage of surface water. In all cases the plants were well mulched with a light layer of hay to minimize possible winter injury.

Effects on Host Development

In considering the results of this transplanting experiment, it should be kept in mind that all the seedings were made on a single date and that the age and development of the plants therefore were different at each successive transplanting. The seed was sown, however, on a desirable planting date for winter wheat in southern Wisconsin, and the development of the plants thus approximated that occurring under field conditions. The first four transplantings were made at intervals of three to four days. Beginning October 16 they were made at weekly intervals until April 4, but the results presented include only those to December 4. The early fall period proved to be the important period, and the presentation of only the early data is adequate. The data obtained in this experiment are given in Table 3 and the curves appear in Figure 4.

Table 3.—*Influence of the duration of exposure to infection and the stage of seedling development, in relation to daily fluctuating soil temperatures, on the development of the rosette phase of the mosaic disease of Harvest Queen wheat, as determined by transplanting seedlings from or to naturally infested soil at different dates during the fall of 1923, as all seedings made October 3*

Date of transplanting	Age of plants from date of seeding (days)	Stage of development	Period	Average soil temperatures (° C.)			Percentage of plants showing rosette when transplanted from—	
				Mean	Maximum	Minimum	Noninfested soil to infested soil	Infested soil to noninfested soil
Oct. 5	2	Kernel swollen but not germinated	Oct. 5–7				100	0
Oct. 8	5	Coleoptile 3 to 4 mm. long	Oct. 8–11	14.0	18.3	9.7	95	0
Oct. 12	9	Plumule still enclosed; coleoptile 6 to 13 mm	Oct. 12–15	10.8	14.3	7.2	90	0
Oct. 16	13	1-leaf to 2-leaf stage, mostly 1 plumule 37 to 62 mm., secondary roots starting on few.	Oct. 16–22	8.0	11.0	5.0	25	0
Oct. 23	20	2-leaf stage	Oct. 23–29	6.4	9.1	3.8	10	42.9
Oct. 30	27	3-leaf stage	Oct. 30–Nov. 5	3.0	4.5	1.5	0	75.0
Nov. 6	34	Coleoptile loosening and tillers appearing in about half the plants.	Nov. 6–12	3.4	6.2	.6	0	77.8
Nov. 13	41	4-leaf stage; 1 to 2 tillers appearing in all plants	Nov. 13–19	4.8	6.6	3.0	0	76.5
Nov. 20	48	do	Nov. 20–26	2.6	3.5	1.7	0	95.0
Nov. 27	55	do	Nov. 27–Dec. 3	.3	.9	−.3	0	95.0
Dec.* 5	63	do	Dec. 4–10	−.3	.6	−1.1	0	95.0

* Transplantings were continued weekly from Dec. 5 to Apr. 4, with the following results: Seedlings transplanted from noninfested to infested soil did not show any rosette, whereas those transplanted from infested to noninfested soil consistently showed 95 per cent rosetted plants.

Effects on Disease Development

In the series in which the plants originally growing in the infested soil were transplanted to the disease-free soil, no rosette occurred in any of the first four transplantings. The absence of symptoms in the

plants which grew in infested soil for as long as 13 days from the date of seeding was very striking. When transplanted, the seedlings were in the 1-leaf to 2-leaf stage, and secondary roots were starting on a few. Certainly the soil temperature was favorable during this period, and the duration of exposure to infection could hardly explain the early lag and the sudden rise in the disease curve. The type of disease curve obtained suggests the possibility that the effect of the mercury in the 1:1,000 bichloride-of-mercury solution used in the surface sterilization of the underground parts may be responsible for this relation. Considering the disease curve further, it is seen that approximately 43 per cent of the plants were rosetted in the next weekly transplanting, October 23. The percentages increased to

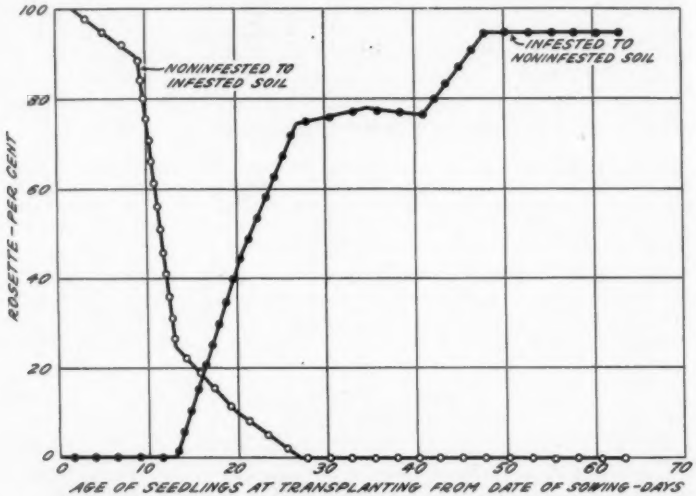

FIG. 4.—Influence of the duration of exposure to infection and of the stage of seedling growth on the development of the mosaic disease in Harvest Queen wheat under field conditions, as expressed in terms of rosette in seedings made on October 3, 1923, a date suitable for field sowing, when seedlings were transplanted to or from infested soil at different intervals

from 75 to 80 per cent in the next three weekly transplantings, but it was not until the November 20 transplanting, or after a period of 48 days' exposure to infested soil, that the maximum percentage of the plants, 95 per cent, showed the rosetted condition. In all transplantings after this date, 95 per cent of the plants consistently showed the rosetted condition. In no case did any of the plants in the control transplantings show symptoms of rosette. (See fig. 5.)

For the series involving transplantings in the opposite direction, namely, from disease-free or control soil to infested soil, it is seen that the disease curve is a rather steep one. For the first transplanting, consisting of swollen kernels, the rosetted plants represent 100 per cent of the total. They decrease in percentage slightly in the next two transplantings, and decline rapidly to 25 per cent in the fourth (October 16) transplanting. In the October 23 transplanting, with seedlings in the 2-leaf stage, only 10 per cent of the plants developed rosette, and in the October 30 transplanting, with seedlings in the

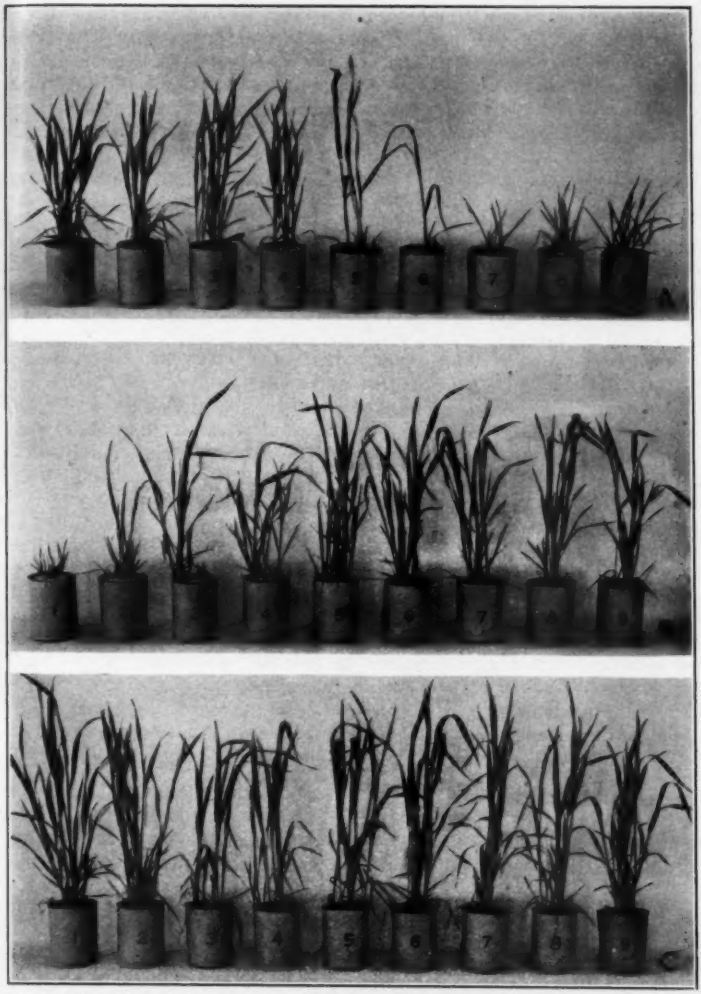

FIG. 5.—Harvest Queen wheat plants from the 1923-24 transplanting experiment, the data for which are given in Table 3: A, Seedlings transplanted from infested to noninfested soil; B, seedlings transplanted from noninfested to infested soil; C, seedlings transplanted from noninfested to noninfested soil (controls). In each of the corresponding series, A, B, and C, the transplantings were all made on the following dates, respectively: No. 1, October 5; No. 2, October 8; No. 3, October 12; No. 4, October 16; No. 5, October 23; No. 6, October 30; No. 7, November 6; No. 8, November 13; No. 9, November 20. Note the increasing appearance of rosette in the late transplantings in series A, and in the early transplantings in series B, and also the absence of rosette in the controls, series C. (Photographed by H. H. McKinney)

3-leaf stage, none of the plants showed rosette. No indications of rosette occurred in any of the subsequent transplantings. (See fig. 5.) It must be remembered, however, that the mean weekly soil temperature was gradually declining and at the last-mentioned date of transplanting it was near the point where growth activities cease. There-

fore the curve for the disease development (fig. 4) can not be analyzed too critically, for, as the seedlings were becoming older with each successive transplanting, the soil temperature at the same time was gradually declining. Two variable factors thus were involved, namely, seedling age and soil temperature, both of which are important and each of which influences and possibly may obscure the effect of the other.

FIELD TRANSPLANTING EXPERIMENTS, 1924-25

The experiment conducted during 1924–25 was a continuation and expansion of that of 1923–24 and was designed to permit analysis of the individual effects of certain factors which were operating together in the previous experiment. These factors include the period of exposure to infection and the root sterilization with $HgCl_2$, on the one hand, and the stage of seedling development and the environmental conditions, on the other. Both the Harvest Queen and the Currell varieties were used in this experiment.

Beginning August 4, seedings of each variety were made at weekly intervals until November 11, in infested and in disease-free soil. The grain was sown in 10-inch earthen pots, the pots were sunk in the ground after planting, and the seedlings were transplanted as indicated. The seedlings were watered periodically before and after transplanting. Four transplantings were made, September 16, October 7, October 27, and November 18, respectively. Each transplanting involved seedlings of 6 different ages, expressed in weeks, namely, 6, 5, 4, 3, 2, and 1. The methods employed and the number of seedlings transplanted from disease-free control soil to infested soil, and vice versa, were the same as in the previous experiment. The seedlings growing originally in infested soil were transplanted both with and without the surface sterilization of the underground parts for 5 minutes in 1:1,000 bichloride of mercury. Approximately 4,000 seedlings were involved in this transplanting experiment.

Effects on Host Developments

The results of this experiment together with the soil-temperature data, are presented in Tables 4 and 5. The development of the seedlings of corresponding ages varied somewhat at the different transplantings. In general, however, the 1-week, 2-week, and 3-week seedlings in each of the four series represented 1-leaf, 2-leaf, and 3-leaf stages, respectively, with only the seminal roots developed. The seedlings in the late series were somewhat smaller than the corresponding ones in the earlier series. The 5-week and 6-week plants of the first series produced a very rank top growth on which leaf rust was prevalent. As a result of the severe rust infection and the high soil and air temperatures, these plants were pale green. The leaves in many cases were yellow, and the older ones were dead or dying. The other seedlings of this series were dark green, vigorous, and robust. The seedlings of the successive series presented general growth relations similar to those for the first series. The rust infection became less on the old plants of the next two transplantings and was absent in the last one. The top growth of the corresponding seedlings decreased more or less with the successive transplantings and the root systems became relatively larger. Helminthosporium infection was

especially pronounced on the basal structures of plants growing in infested soil and developing at relatively high soil temperatures.

Considerable variation occurred in the development of tillers and subcoronal internodes in each variety; but, on the whole, Harvest Queen showed a tendency to produce more tillers, and Currell showed a tendency to develop more pronounced subcoronal internodes. The Harvest Queen seedlings were very hardy and generally survived the severe winter conditions better than the Currell seedlings. In the case of both varieties, the percentage of sterilized seedlings which were winterkilled was greater than that of the unsterilized seedlings.

TABLE 4.—*Influence of the period of exposure to infection and of surface disinfection of transplanted seedlings, in relation to daily fluctuating soil temperatures, on the development of the mosaic disease in Harvest Queen and Currell wheats, as determined by transplanting seedlings of six different ages from naturally infested to noninfested soil on each of four different dates during the fall of 1924*

Dates of sowing	Average soil temperature in the week following sowing (° C.)			Time elapsed between sowing and emergence (days)	Period of exposure to infested soil (weeks)	Date of transplanting	Development of mosaic disease (per cent) in—					
							Harvest Queen				Currell	
	Mean	Maximum	Minimum				Mottling [a] when sterilized	Mottling [a] when not sterilized	Rosette when sterilized	Rosette when not sterilized	Mottling [a] when sterilized	Mottling [a] when not sterilized
Aug. 4	22.9	26.4	19.5	4	6	Sept. 16	45 D	14 D	0	0	50.0 D	75.0 D
Aug. 11	23.1	28.7	17.4	5	5		71 D	18 D	0	0	57.9 D	35.0 D
Aug. 18	21.3	25.0	17.6	4	4		100 D	19 D	0	0	75.0 D	35.7 D
Aug. 25	21.8	25.7	17.8	4	3		92 D	14 D	8.3	0	64.3 D	20.0 D
Sept. 1	15.8	18.8	12.9	6	2		43 D	90 C	14.3	50.0	72.2 C	47.4 C
Sept. 8	12.7	14.6	10.7	6	1		40 D	100 C	0	66.7	43.8 C	70.6 C
Aug. 25	21.8	25.7	17.8	4	6	Oct. 7	71 C	40 D	42.9	40.0	37.5 C	25.0 D
Sept. 1	15.8	18.8	12.9	6	5		50 C	91 C	50.0	63.6	72.2 C	64.7 C
Sept. 8	12.7	14.6	10.7	6	4		100 C	100 C	[b]	83.3	85.7 C	94.1 C
Sept. 16	14.8	18.0	11.5	5	3		67 C	37 C	20.0	[c]70.2	66.7 C	46.7 F
Sept. 22	13.3	16.3	10.4	6	2		92 C	71 C	30.8	57.1	43.8 M	41.2 M
Sept. 29	10.7	12.8	8.5	6	1		44 D	30 D	0	0	31.3D	25.0 D
Sept. 16	14.8	18.0	11.5	5	6	Oct. 27	38 C	93 C	37.5	53.3	73.3 C	53.3 D
Sept. 22	13.3	16.3	10.4	6	5		40 C	80 C	50.0	60.0	72.2 C	55.0 M
Sept. 29	10.7	12.8	8.5	6	4		80 C	100 C	53.3	42.1	86.7 C	63.2 M
Oct. 7	10.3	11.7	8.9	8	3		58 C	100 C	50.0	42.9	87.5 C	58.3 C
Oct. 13	10.1	11.5	8.7	8	2		50 D	18 D	0	0	55.6 D	27.1 D
Oct. 20	5.4	6.5	4.3	8	1		25 D	42 D	0	0	50 D	46.2 D
Oct. 7	10.3	11.7	8.9	8	6	Nov. 18	0	100 C	0	0	100 C	50.0 C
Oct. 13	10.1	11.5	8.7	8	5		0	73 D	0	0	33 C	37.5 C
Oct. 20	5.4	6.5	4.3	8	4		[d]	------	[b]	[d]	[d]	[d]
Oct. 27	6.1	7.8	4.4	7	3		0	------	0	[d]	[d]	[d]
Nov. 4	3.1	4.7	1.6	8	2		[d]	------	0	[d]	[d]	[d]
Nov. 11	2.0	2.9	1.1	[e]	[e]	[e]	------	------	------	------	------	------

[a] Mottling symbols: D=Doubtful, F=faint, M=midmotley, C=conspicuous.
[b] All plants winterkilled after transplanting.
[c] Only a few plants survived, and the percentage was obtained by using the average of percentages from the next older and next younger seedlings.
[d] One plant survived and showed unmistakable mottling.
[e] All seedlings winterkilled before emergence.

EFFECTS ON DISEASE DEVELOPMENT

From the results of the experiments with the Harvest Queen seedlings subjected to the sterilization treatment, which are shown in Table 4 and graphically presented in Figure 6, it will be seen that the rosetted condition developed quite differently and that the interval of expo-

sure, the stage of seedling development at the time of transplanting, and the soil temperature were important factors influencing this condition. In the 6-week, 5-week, and 4-week seedlings transplanted September 16 it is interesting to note that no rosette occurred. The seedlings developed at mean weekly soil temperatures of 21° C. and above. Even the minimum soil temperatures of 17° to 19° C. for short periods each day were not sufficient to permit infection. These results agree well with those in the studies under controlled soil temperatures. The 3-week and 2-week transplanted seedlings showed only a small number of rosetted plants, 8.3 and 14.3 per cent, respectively, and the 1-week seedlings showed no cases of typical rosette. One plant, however, showed slightly suspicious indications.

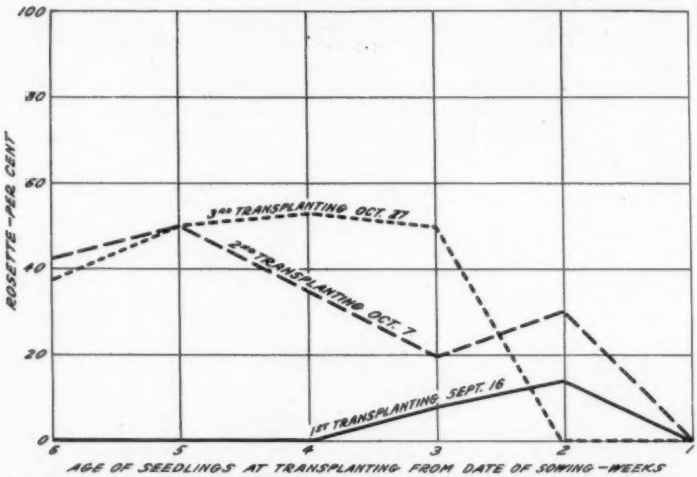

FIG. 6.—Influence of the period of exposure to infection on the development of rosette in Harvest Queen wheat under field conditions, as determined by transplanting seedlings of different ages from infested to noninfested soil on different dates during the fall of 1924. The surface of underground seedling parts were disinfected for 5 minutes in 1:1,000 bichloride of mercury immediately preceding transplanting

In the second transplanting, October 7, all of the seedlings except the 1-week series showed rosette, and the percentages were higher than those of the corresponding seedlings of the first transplanting. The greatest number of diseased plants, 50 per cent, occurred in the 5-week stage, followed in order by the 6-week, 4-week, 2-week, and 3-week stages. The mean weekly soil temperatures ranged from 10.3° to 2° C.

The third series, transplanted October 27, showed almost 50 per cent of rosette in the 5-week, 4-week, and 3-week seedlings. The 6-week seedlings had slightly fewer diseased plants than the above mentioned seedlings. It is of interest to note that neither the 2-week nor the 1-week seedlings showed definite rosette. In the 2-week stage 10 per cent of the seedlings looked a little questionable, but none of the 1-week seedlings showed any indications of rosette. These seedlings, it must be remembered, developed at lower soil temperatures than the older seedlings of the series, and furthermore their time interval of exposure to infection was much shorter.

The rosette data for similar seedlings not receiving the surface sterilization of underground parts also are given in Table 4 and the data are presented as curves in Figure 7. In general, the percentages of rosetted plants are higher in the unsterilized seedlings than in the sterilized seedlings, especially in the case of the younger ones. These results indicate that the $HgCl_2$ sterilization either is destroying or inactivating the causal agent in some way or is interfering with its subsequent development or activity in the plant. This, then, would tend to explain the lag in the early part of the disease curve for the 1923–24 experiments. The sterilized and unsterilized plants showed no visible variations in their development, and in view of this similarity there is a suggestion that the $HgCl_2$ possibly may be exerting its effect more on the causal agent than on the host.

In the first transplanting, September 6, the 6-week, 5-week, 4-week, and 3-week seedlings did not show any signs of the rosetted condition.

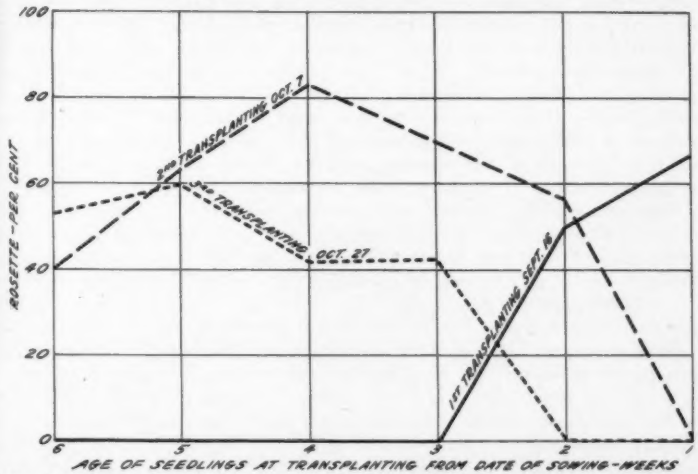

FIG. 7.—Influence of the period of exposure to infection on the development of rosette in Harvest Queen wheat under field conditions, as determined by transplanting seedlings of different ages from infested to noninfested soil on different dates during the fall of 1924, when no surface disinfection of underground parts was given

These results agree well with the soil-temperature relations previously considered. The relatively large number of rosetted plants occurring in the 1-week and 2-week seedlings, 67 and 50 per cent, respectively, is very striking, as compared with the values for the corresponding sterilized seedlings, 0 and 14 per cent, respectively. Even though the 2-week seedlings were exposed to infested soil one week longer than the 1-week seedlings, the latter had a higher percentage of disease. An examination of the soil-temperature data, shows that the 1-week seedlings experienced a slightly lower and more favorable soil temperature than the 2-week seedlings. Under these conditions it appears that the soil-temperature factor is more important than the period of exposure. It appears further that, under favorable conditions, an extremely high percentage of the disease may develop when the seedlings have grown in infested soil for a period of only one week from date of seeding.

The plants from the next transplanting, October 7, show high percentages of disease in all cases except the 1-week seedlings. The highest percentage, 83, occurred in the 4-week stage, followed closely by the 3-week, 5-week, and 2-week stages. A considerable decrease occurred in the 6-week seedlings, and no indications of disease occurred in the 1-week seedlings.

In the third transplanting, October 27, very similar effects were obtained in the 6-week, 5-week, 4-week, and 3-week seedlings, namely, 42 to 60 per cent. Neither the 1-week nor 2-week seedlings showed any indications of disease.

In the fourth transplanting, November 18, no indications of rosette were observed, although only a relatively few seedlings survived the winter.

A noteworthy point is brought out by results of the experiment with the seedlings of the October 7 sowing used in the third and the fourth transplantings. From Table 4 it will be seen that 43 to 50 per cent of the plants in the third transplanting showed rosette, whereas plants from the same sowing in the fourth transplanting did not show the disease. Only nine plants of the last transplanting survived the winter, so that the number is too small to permit of any conclusions. However, it appears that either the infected plants were more easily winter injured and that all were killed, or else that the development of the disease was interfered with in some way by the transplanting of the host at the extremely late date, November18. In this connection it may be said that regardless of the increased period of exposure to infested soil, the oldest seedlings of a particular transplanting did not show the maximum development of rosette. Younger seedlings exposed to infection for even shorter time intervals had higher percentages of rosette. The age of the seedling at the time of transplanting, therefore, appears to be a contributing factor.

Thus far, only the rosetted condition of Harvest Queen seedlings growing in infested soil has been considered. The mottling phase likewise has been studied in all cases. But as pointed out earlier, the leaf mottling is a very variable manifestation and one extremely difficult to express. The mottling data for the Harvest Queen seedlings growing in infested soil are included also in Table 4, but no disease curves are presented.

In both the sterilized and unsterilized transplanted seedlings, it may be said that variable leaf mottling generally occurred throughout the entire experiment. The mottling was so faint as to be questionable in most cases on the older seedlings of the first transplanting, and high percentages of conspicuous mottling occurred only in the 1-week and 2-week unsterilized seedlings. This is the only outstanding instance in which the surface sterilization of the seedlings appears to have interfered with the mottling development somewhat as it appeared to do with the rosette development. Conspicuous mottling generally was obtained in the subsequent transplantings, with the exception of somewhat consistently questionable mottling in the youngest transplanted seedlings. In cases in which the mottling was so faint as frequently to be questionable, no rosette occurred. Unsterilized seedlings of the fourth transplanting showed excellent mottling in one case and questionable mottling in the other. No cases of rosette occurred in this transplanting. It appears, therefore,

that mottling may occur under a wider range of conditions than rosette.

While there were no consistent differences in the mottling of the sterilized and unsterilized series, the mottling curves for the different transplantings appeared to be more consistent and uniform in the case of the sterilized seedlings than in the case of the unsterilized seedlings. Reference to Table 4 will reveal the mottling data for the Currell variety. They are very similar to those just considered for the Harvest Queen variety and need not be considered further. It should be pointed out, however, that conspicuous mottling in varying percentages was obtained in the 1-week and 2-week seedlings in both the sterilized and unsterilized series of the first transplanting and in all the surviving seedlings of the last transplanting.

Simultaneously with the different transplantings of seedlings from infested soil to disease-free soil, corresponding transplantings of both

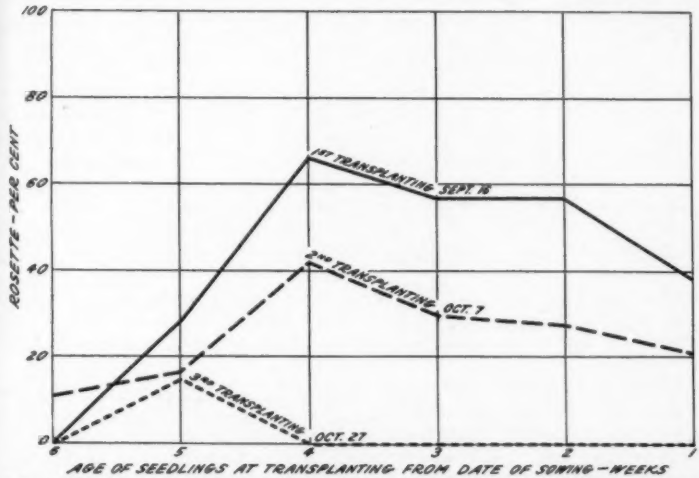

FIG. 8.—Influence of stage of seedling growth on the development of rosette in Harvest Queen wheat under field conditions, as determined by transplanting seedlings of different ages from noninfested to infested soil on different dates during the fall of 1924

varieties were made in the opposite direction. The results of this study are included in Table 5 and the curves appear in Figure 8.

If the rosette phase in the Harvest Queen variety is considered first, it will be seen that the disease generally developed to the greatest extent in the different seedlings of the first transplanting (September 16), followed uniformly by those of the second transplanting (October 7). The curves are remarkably uniform and consistent. In fact, the only noticeable difference is that, whereas no rosette developed in the 6-week seedlings of the first transplanting, a relatively small percentage developed in the corresponding seedlings of the second transplanting.

The disease curves further demonstrate the influence of soil temperature, and agree well with the results obtained in the controlled and uncontrolled soil-temperature experiments. While age of seedling and soil temperature are important factors influencing infection,

neither apparently modifies or obscures the effect of the other under the experimental conditions employed. In both the first and second transplantings, the seedlings 4 weeks old at the time of transplanting showed the highest percentage of rosette, followed in order by the 3-week, 2-week, 1-week, 5-week, and 6-week stages.

TABLE 5.—*Influence of stage of seedling development, in relation to daily fluctuating soil temperatures, on the development of the mosaic disease of Harvest Queen and Currell wheats, as determined by transplanting seedlings of six different ages from noninfested to naturally infested soil, on each of four different dates, during the fall of 1924*

Date of sowing	Age of seedlings when transplanted	Date of transplanting	Average weekly soil temperatures for six successive weeks after transplanting				Development of mosaic disease (per cent) in—		
			Period	Mean	Maximum	Minimum	Harvest Queen		Currell, mottling
							Rosette	Mottling	
	Weeks			° *C.*	° *C.*	° *C.*			
Aug. 4	6	Sept. 16	Sept. 15–21	14.8	18.0	11.5	0	[a] 8.0	46.2
Aug. 11	5		Sept. 22–28	13.3	16.3	10.4	28.6	71.0	50.0
Aug. 18	4		Sept. 29–Oct. 5	10.7	12.8	8.5	66.7	67.0	50.0
Aug. 25	3		Oct. 6–12	10.3	11.7	8.9	57.1	71.0	55.0
Sept. 1	2		Oct. 13–19	10.1	11.5	8.7	57.1	50.0	11.8
Sept. 8	1		Oct. 20–26	5.4	6.5	4.3	38.1	10.0	17.6
Aug. 25	6	Oct. 7	Oct. 6–12	10.3	11.7	8.9	11.1	33.0	33.3
Sept. 1	5		Oct. 13–19	10.1	11.5	8.7	16.7	60.0	31.6
Sept. 8	4		Oct. 20–26	5.4	6.5	4.3	42.1	68.0	41.2
Sept. 16	3		Oct. 27–Nov. 2	6.1	7.8	4.4	30.1	80.0	35.0
Sept. 22	2		Nov. 3–9	3.1	4.7	1.6	27.8	50.0	5.0
Sept. 29	1		Nov. 10–16	2.0	2.9	1.1	21.1	53.0	24.0
Sept. 16	6	Oct. 28 [b]	Oct. 27–Nov. 2	6.1	7.8	4.4	0	15.0	[c] 10.0
Sept. 22	5		Nov. 3–9	3.1	4.7	1.6	15.0	40.0	10.5
Sept. 29	4		Nov. 10–16	2.0	2.9	1.1	0	10.0	20.0
Oct. 7	3		Nov. 17–23	.1	.9	−.7	0	0	13.0
Oct. 13	2		Nov. 24–30	−3.5	−2.8	−4.2	0	[a] 5.0	20.0
Oct. 20	1		Dec. 1–7	[d]	[d]	[d]	0	0	0
Oct. 7	6	Nov. 18 [b]	Nov. 17–23	.1	.9	−.7	0	[a] 6.0	[e]
Oct. 13	5		Nov. 24–30	−3.5	−2.8	−4.2	0	0	[e]
Oct. 20	4		Dec. 1–7	[d]	[d]	[d]	0	0	[f]
Oct. 27	3		Dec. 8–14	[d]	[d]	[d]	[e]	[e]	[f]
Nov. 3	2		Dec. 15–21	[d]	[d]	[d]	[e]	[e]	[f]
Nov. 11	1		Dec. 22–28	[d]	[d]	[d]	[e]	[e]	[f]

[a] Mottling so faint as to be questionable.
[b] The regular date of transplanting would have been a day earlier, and therefore the temperature record for the week would have been begun one day earlier than the date of transplanting.
[c] Mottling faint.
[d] Soil thermograph out of order.
[e] Plants winterkilled after transplanting.
[f] Plants killed before emerging.

Whether the 4-week seedlings actually are more susceptible to infection than the seedlings of other ages, or merely represent the best stage for transplanting, is not known. The fact that in only one instance a small percentage of rosette developed in the transplanted 6-week seedlings indicates that little or no infection is likely after a period of 5 or 6 weeks from date of seeding. The decline in the rosette curve with the 1-week seedlings very probably is due to the diminution of surface exposed to the infested soil, caused by the smaller root system. In addition, the setback of transplanting very probably is greater with these than with older seedlings. However, no perceptible retardation was noted.

In the third transplanting, the only cases of rosette occurred in the 5-week seedlings, 15 per cent showing the disease. The mean weekly soil temperature for the five successive weeks after transplanting ranged from 6.1° to −3.5° C. The fact, then, that the soil temperature was so relatively low and that the transplanted seedlings grew under such unfavorable conditions, probably explains the absence of the disease.

In the fourth transplanting, seedlings of the three latest sowings were winterkilled soon after emergence. A few plants of the 6-week, 5-week, and 4-week seedlings survived, but none of these showed any indications of rosette. The soil temperature ranged from 0.1° C. to considerably below −4.2° C. after the seedlings had been transplanted to infested soil.

From an examination of the mottling data for the Harvest Queen variety in Table 5, it is evident that mottling occurred in each of the different ages of seedlings of the first two transplantings. The two disease curves, though not shown in this paper, are very similar, the highest percentages of mottled plants occurring in the 3-week, 4-week, and 5-week seedlings and diminishing in younger or older seedlings. The mottling was questionable in the oldest seedlings of the first transplanting and it was either absent or very faint in the younger seedlings of the third transplanting.

It is interesting to note that a small percentage of faint mottling occurred in the oldest seedlings of the fourth transplanting, even though the soil temperature ranged from 0.9° C. to below 0° C. after the transplanting to infested soil.

In general, the percentages of plants showing mottling were higher than those showing rosette, and in cases in which the mottling was either absent or faint no rosette occurred. It thus appears that the leaf-mottling condition is capable of developing over a wider range of both soil temperatures and seedling ages than is the rosette condition.

The leaf-mottling data for the Currell variety also are shown in Table 5. Higher percentages of mottling occurred in the seedlings of the first transplanting, followed in order by those of the second and third transplantings. Mottling was very uniform with the 3-week, 4-week, and 5-week seedlings in each transplanting, and a noticeable decrease appeared in the younger seedlings. No mottling was evident in the 1-week seedlings of the third transplanting. All seedlings of the fourth transplanting were winterkilled, and no mottling data were obtainable. In comparing the leaf-mottling data of Currell with those of Harvest Queen, it is evident that the percentages generally are less and that the disease curves, though not shown in this paper, differ somewhat. Nevertheless, the soil-temperature and seedling-stage ranges are about the same for each variety.

DISCUSSION

In diagnosing the mosaic disease of winter-wheat seedlings, macroscopical symptoms have been employed as criteria. Microscopical symptoms, such as intracellular bodies, offer a more delicate diagnosis in extreme or doubtful cases, but the necessity of studying large numbers of plants makes this method slow and laborious. Until the presence of a causal agent can be detected, the mosaic disease

of wheat, like all other virus diseases, must be defined in terms of host response. Obviously, the presence of symptoms always indicates that infection has occurred, but the absence of such symptoms does not necessarily indicate that infection has not occurred.

As a rule, the rosette phase is a generally constant and unmistakable index, and its occurrence can be effectively expressed in terms of percentages. Intermediate stages may occur in some instances, but they have been the exception in the experiments reported. The leaf mottling, on the other hand, is a much more difficult response to calibrate, inasmuch as both the percentage of plants showing mottling and the intensity of the mottling are variables. The present-day quantitative and qualitative methods of mottling determination, therefore, are unsatisfactory and inadequate, and this condition serves as the explanation for the greater emphasis given to the rosette phase than to the mottling phase throughout this paper.

Winter-wheat seedlings are particularly hardy and may withstand much rough treatment with no visible injury. These properties, therefore, well adapt them for experimentation of this type. The method of transplanting, of course, is open to criticism, for it necessitates the removal of the seedlings from the soil and causes considerable disturbance. However, at the time of these experiments, this method was the most natural and the only one applicable to the problem. It is realized that growth, or the ability of the tissues to resume growth after transplanting, is an important matter and that all factors governing growth before and after transplanting exert important influences. The transplanting results, therefore, must be accepted with these points in mind.

In general, the results presented in this paper indicate that the seedling infection and symptom expression of the mosaic disease in the Harvest Queen and Currell varieties are most pronounced under those environmental conditions which are best adapted for the development of the host during the seedling stages. Conditions, therefore, which promote high resistance to many plant diseases promote high susceptibility to the wheat mosaic disease. In this respect the wheat disease agrees well with the other known virus diseases of the mosaic type, as it has long been known and well established that the mosaic diseases occur and develop best with the most active host development. Similar relations, moreover, recently have been reported by Keitt (*7*), by Jones (*6*), and by Fellows (*2*), for certain plant diseases outside of the virus category.

The data herein presented certainly demonstrate that soil temperature and soil moisture are important factors influencing infection and subsequent development of the mosaic disease of winter wheat. The fact that the disease developed only at low soil temperatures and decidedly best at high soil moistures is particularly significant. No doubt the effect of one or both of these two environmental factors is important in the frequent development of the disease in patchy and more or less circular areas under field conditions. However, the possible variation of soil infestation is a question that always must be considered.

The absence of rosette and mottling in seedlings developed at the relatively high soil temperatures of 23° and 30° C. and at low soil moistures brings up interesting questions at this time. What is the

explanation of these relations? Is it a question of either host response or virus activity, or both? Or, if it is purely a question of host response, is the resistance which is developed during an early seedling stage dominant throughout the susceptible seedling stage? Unfortunately, the information now available concerns only the disease expressed in terms of host response. Nothing is known concerning the virus response, and until data are obtained from property studies made on the virus in the soil, in the expressed tissue fluids, and isolated from these respective media, little can be offered in the way of a satisfactory explanation.

Different types of metabolism within the host occur at the low and the high soil-temperature extremes. These relations are very significant and undoubtedly play an important part in infection and the subsequent development of this disease. At the low temperatures, emergence is relatively slow, the roots develop first and faster than the plumules, root development is highly stimulated, the roots are fleshy in texture, the elongation of the subcoronal internode is inhibited, and the coleoptile develops faster than the growing culm, thereby protecting the growing tip. It is in the seedlings of this type and under the corresponding environmental conditions essential for their development that the mosaic disease of wheat develops best. Either the protection offered to the growing point by the coleoptile possesses no value in the case of this disease, contrary to its rôle in certain other soil-borne diseases, or else no infection occurs through this structure. Conversely, at the higher temperatures, emergence is relatively speedy, the plumules develop first and faster than the roots, root development generally is depressed, the roots are fine and fibrous, brown in color, and not fleshy in texture, the subcoronal internode is greatly stimulated, and the growing culm generally ruptures the coleoptile before emergence. It is in the seedlings of this type, and under the corresponding environmental conditions essential for their development, that the mosaic disease of wheat fails to develop.

From the standpoint of the causal agent, the results would seem to indicate that the absence of rosette and mottling at the higher soil temperatures of 23° C. or above was not due entirely to the temperature effect on the virus. If the virus is as sensitive as the most sensitive virus known to-day, namely, the virus causing the cucumber mosaic, it would be able to withstand temperatures approaching 50° to 60° C. for short periods. However, too much emphasis should not be placed on a comparison of the results of such property studies; for, unless the experiments are conducted under the same or similar conditions and in the same or similar medium, the results possess little in the way of significance.

It is difficult also to believe that the absence of rosette and mottling can be explained entirely on the basis of a desiccating effect on the virus at the low soil moistures under favorable temperatures. Infested soil from Granite City, Ill., has been stored in a highly desiccated state over a period of four years and when sown to wheat has produced rosette and mottling most generally and severely.

While only the questions of host and causal agent have been considered thus far, it is possible also that some soil vector of an animal nature is active in the promotion of mosaic disease and that the different results may be due to the various responses of the disease

to the experimental conditions. So far, however, there is no evidence of such a vector.

Because the rosette phase of the mosaic disease of winter wheat is controlled completely by the use of resistant and immune varieties, as shown in previous papers (*3*, *8*, *13*), no recommendation for the control of the disease on a basis of date of seeding is made. In extremely early and late fall sowings, in cases where the disease is partially or completely controlled, the seeding dates are so far removed from the normal seeding dates in the field that they would be impracticable.

SUMMARY

The occurrence and subsequent development of the mosaic disease of winter wheat is closely associated with normally active growth of the seedling.

The period of infection is limited to the early seedling stage. Under favorable environmental conditions, an interval of one week from date of seeding is sufficient to permit a high percentage of rosetted or mottled plants. Where the environmental conditions were less favorable, mottling occurred at time intervals too short for the development of rosette.

The disease developed only within a relatively restricted soil-temperature range. In a test at the four different constant soil temperatures, 10°, 16°, 23°, and 30° C., the disease occurred only at 10° and 16° C. The leaf mottling of the Currell variety occurred to about an equal degree at each of the two soil temperatures, while the rosette and the leaf mottling of the Harvest Queen variety developed best at 16° C. No indications of either rosette or mottling appeared in either variety at the two higher soil temperatures.

A high soil-moisture content decidedly favored the occurrence of both rosette and leaf mottling, whereas a medium soil-moisture content was proportionately less favorable. No indication of symptoms appeared under the low soil-moisture contents.

Under the controlled conditions described, no shifts occurred in the soil-temperature and soil-moisture optima when the value for either factor was altered.

Rosette developed under field conditions at soil temperatures strikingly similar to those found most favorable in the controlled tanks. Faint mottling of both varieties, however, developed at somewhat higher soil temperatures under these fluctuating conditions.

In the case of seedlings transplanted from infested soil to uninfested soil, the age of the seedling at the time of transplanting appeared to be a factor influencing the development of the disease; that is, the percentage of diseased plants increased only within limits for increased periods of exposure to infested soil.

The rosette-disease infection curves for the seedlings of six different ages varied with the transplantings on different dates. These relations are in direct line with those obtained in the tank and the field soil-temperature experiments, the disease developing only at or below 16° C.

The susceptibility of the seedlings to rosette is influenced by the stage of seedling development. In general, the 4-week seedlings appeared most susceptible, followed in order by the 3-week, 2-week, 1-week, 5-week, and 6-week seedlings, respectively. Only in one

instance was the disease obtained in the 6-week seedlings, and the percentage then was very small.

For both varieties tested, mottling was obtained in each of the six different ages of seedlings. In contrast to rosette, the mottling appeared prominently in the 6-week seedlings.

Comparing the different varietal responses, it is evident that mottling was more general than rosette, and that the Harvest Queen variety showed higher percentages of mottled plants than the Currell variety.

Surface sterilization of the subterranean parts of infected seedlings with bichloride of mercury at the time of transplanting noticeably reduced the occurrence of rosette, especially in the younger seedlings. This treatment entirely prevented the occurrence of rosette in the 1-week seedlings.

Leaf mottling was not similarly affected by the surface sterilization. On the contrary, the percentages of mottling frequently were higher and noticeably more uniform in the sterilized than in the unsterilized series.

The evidence obtained does not throw any definite light on the nature of the causal agent.

Any possible causal relation of *Helminthosporium sativum* naturally occurring in the infested soil to either the rosette or mottling phases of this disease is further disproved.

No effective control measures for this disease are practicable on the basis of seeding date, as suggested by the soil-temperature relations.

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FACTORS AFFECTING THE POPPING QUALITY OF POP CORN[1]

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INTRODUCTION

Only during the last half century has corn popping developed into an industry of commercial importance. The resulting demand for pop corn has given the stimulus necessary to the growing of the crop in a large way and has expanded the acreage very considerably.

The quality of pop corn depends upon its palatability or flavor and upon its popping expansion. Although it is commonly recognized that great differences exist in the "poppability" of different varieties and in various lots of the same variety, the matter seems not to have been studied in a critical way.

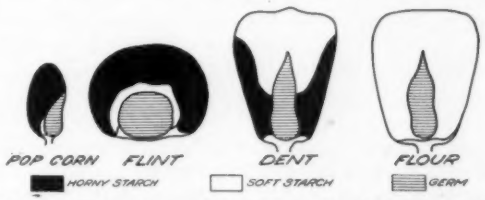

FIG. 1.—Diagrammatic representation of location and relative proportions of horny starch and soft starch in the four main classes of starchy corn

All starchy corns may be placed in one of the four classes—pop corn, flint corn, dent corn, or flour corn—on the basis of distribution and relative proportions of hard and soft starch in the endosperm. The endosperm of the best strains of pop corn is corneous throughout or contains only a small core of soft starch near the center. (Fig. 1.) In flint corn the endosperm consists of a small quantity of soft starch, near the embryo, completely surrounded by corneous starch. In dent corn the corneous starch is confined mainly to the sides of the kernels, soft starch constituting a larger proportion than in flint corn. In flour corn the endosperm is entirely of soft starch except for vestigial traces of hard starch.

The popping properties of the various types of corn follow rather closely the relative proportions of hard or corneous starch in the endosperm. Pop corn, with the greatest proportion of hard starch of any of the four types, is far better than the others in poppability. Flint corn may pop poorly to fairly well, depending on the strain. Dent corn rarely will pop at all, though occasionally a few kernels will pop feebly. Flour corn will not pop at all.

It is generally conceded that the popping process is due to the sudden liberation of pressure produced by steam generated within the kernel.[2] The source of the steam is the moisture contained in

[1] Received for publication July 11, 1927; issued November, 1927. This paper is a report of cooperative investigations made by the Bureau of Plant Industry, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station. Department of Agronomy contribution No. 169.

[2] WEATHERWAX, P. THE POPPING OF POP CORN. Ind. Acad. Sci. Proc. 1921: 149-153. 1922.

the kernel and perhaps to a limited extent, also, water of constitution produced by partial breaking down of the starch molecule when heated.[3] The confining structure, according to Weatherwax, is the colloidal matrix in which the starch grains are embedded within the cell. The success of the explosion in expanding the kernel and completely disrupting its original structure to produce a uniform and tender product depends upon three conditions: (1) Optimum moisture content, (2) an elastic and tenacious confining structure, and (3) proper application of heat.

The first and third of these conditions, viz, optimum moisture content and proper application of heat, may be controlled at the time of popping. The second condition, however, is a property of the endosperm and varies with the sample. It is believed that differences in the poppability of various strains are definitely inherited and that the properties of endosperm structure have a genetic factorial basis, although the mode of inheritance is undoubtedly complicated. As a preliminary step in testing the possibilities of pop corn improvement through breeding, this paper is concerned chiefly with a study of the degree of association between certain morphological kernel characters and popping yield.

EXPERIMENTAL METHODS

With the exception of tests covering the influence of moisture content on poppability, all of the corn used in these experiments was of uniform and probably nearly optimum moisture content. The popping trials were conducted at Washington, D. C., during the winter months. Until the time of popping the samples were stored in an outside shelter protected from direct precipitation but subject to continuous free circulation of air.

The popping trials were made in a wire popper over a gas burner. A wire gauze over the burner helped to give a more uniform distribution of heat. The flame was adjusted so that popping would commence in about one and one-half minutes, which time had previously been found to give the best results. A wire swing for the popper assured a constant distance between the corn and the source of heat.

The index of poppability used in this paper is the ratio of the volume after popping to the volume of the unpopped corn, and for convenience is termed "expansion." Glass graduates were used to measure volumes both before and after popping. A uniform sample 20 cubic centimeters in volume was used throughout for popping, and, except where otherwise stated, the expansion is based on duplicate determinations. The expansion was determined only to the nearest whole number. Besides expansion, six characters of the unpopped kernels were considered, viz, percentage of soft starch, weight of 100 kernels, size as measured inversely by the number of kernels in 25 cubic centimeters, length of kernels, breadth of kernels, and thickness of kernels.

The proportion of soft starch in the endosperm did not lend itself to any simple exact measurement and therefore was estimated. Ten random kernels from each ear were split lengthwise and the halves glued to cardboard. The percentage of soft starch then was esti-

[3] CARR, R. H., and RIPLEY, E. F. WHAT PUTS "POP" IN POP CORN? Ind. Acad. Sci. Proc. 1920: 261-270, illus. 1921.

mated for each kernel separately, and the average of the 10 estimates was taken as the soft-starch content for the ear as a whole. The final ratings were not made until after a preliminary survey had given an intimate acquaintance with the material and had fixed the standards firmly in mind, so that it is thought that the estimates present a very fair basis for purposes of comparison.

Weight of kernels was obtained by weighing 100 kernels from each ear. Another measurement which is highly correlated negatively with weight was also taken, namely, the number of kernels in a measured volume of 25 cubic centimeters. Length of kernel was measured by laying 10 kernels end to end in a groove and dividing the total length in millimeters by 10. Breadth of kernel and thickness of kernel (from germinal to abgerminal face) were obtained similarly.

INFLUENCE OF MOISTURE CONTENT ON POPPABILITY

It is well recognized that the moisture content of pop corn has an important influence on its poppability. Carr and Ripley,[4] however, state that within comparatively wide ranges the moisture content has little effect on popping expansion. Weatherwax,[5] taking a similar view, expresses the opinion that, although maximum, minimum, and optimum moisture contents are indicated, the range is wide. In an exhaustive study on the effect of moisture content and other factors on popping, Stewart [6] concludes that "a moisture content of 13 to 15 per cent appears to be the most favorable for maximum popping yield." He states also that, under New York conditions, outside storage from October to April will give a moisture content approaching the optimum.

TABLE 1.—*Expansion of successive 12-ear samples of White Rice pop corn taken while drying, showing relation between moisture content and poppability*

Sample No.	Moisture (per cent)	Expansion (volumes)	Sample No.	Moisture (per cent)	Expansion (volumes)	Sample No.	Moisture (per cent)	Expansion (volumes)
1	17.0	2	11	11.9	18	21	9.1	11
2	15.6	7	12	11.9	17	22	8.3	9
3	15.6	5	13	11.9	16	23	8.1	6
4	14.2	12	14	10.6	16	24	7.4	7
5	13.6	15	15	10.5	15	25	7.4	8
6	13.6	14	16	10.1	16	26	7.2	6
7	12.7	17	17	10.1	14	27	6.9	8
8	12.7	16	18	9.6	10	28	6.3	7
9	12.6	17	19	9.5	12			
10	12.6	16	20	9.2	11			

A stock of White Rice pop corn grown at Oconomowoc, Wis., was divided into a number of samples of 12 ears each, and successive moisture and popping determinations were made as the samples were drying. Each sample of 12 ears was shelled and thoroughly mixed before testing. The data for these tests are given in Table 1

[4] CARR, R. H., and RIPLEY, E. F. Op. cit.
[5] WEATHERWAX, P. Op. cit.
[6] STEWART, F. C. THE RELATION OF MOISTURE CONTENT AND CERTAIN OTHER FACTORS TO THE POPPING OF POP CORN. N. Y. State Agr. Expt. Sta. Bul. 505, 70 p., illus. 1923.

and are shown graphically in Figure 2. Each value for expansion is the mean of several determinations made from a given 12-ear sample. About 12 per cent appears to be the optimum moisture content for greatest expansion in this sample.

RELATION OF KERNEL CHARACTERS TO POPPABILITY IN WHITE RICE POP CORN

One hundred ears of White Rice pop corn grown, harvested, and cured under as nearly identical conditions as possible formed the material for a preliminary study of the relation of kernel characters to poppability. Data were obtained for the weight of 100 kernels, the number of kernels in a 25 cubic centimeter volume, the percentage

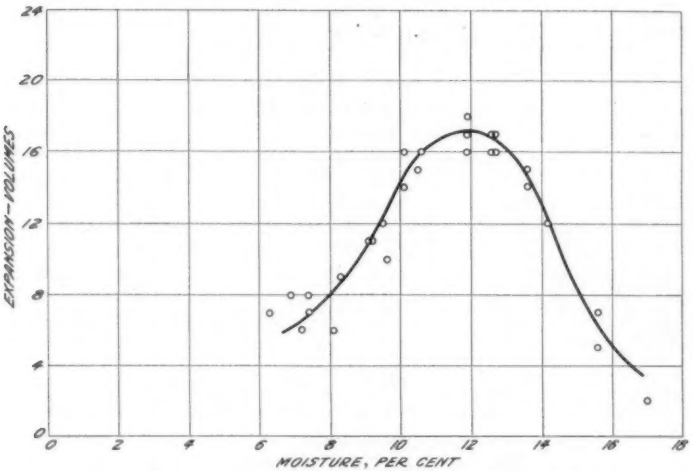

FIG. 2.—Relation between moisture content and popping expansion in White Rice pop corn

of soft starch, and the expansion for each ear. The expansion on popping ranged from 5 to 25 volumes. The ears were grouped into seven classes according to expansion, and the average obtained in each group for the various characters noted is shown in Table 2.

There is a distinct tendency for the size of the kernels to decrease as the expansion increases. In other words, the ears giving the greater increase in volume on popping tend on the average to have smaller kernels. This is true when kernel size is measured either by the weight of 100 kernels or by the number of kernels in 25 cubic centimeters. In group A the average expansion is 6.0, the average weight of 100 kernels is 15.5 gms., and the average number of kernels in 25 cubic centimeters is 130. At the other extreme, in group G, with an average expansion of 24.2, the weight of 100 kernels is only 11.6 gms. and the average number of kernels in 25 cubic centimeters is 177.4.

TABLE 2.—*Relation between certain kernel characters and popping quality of White Rice pop corn, as shown by the mean values of these characters when a population is grouped on the basis of expansion*

[Correlation of expansion with weight of 100 kernels, −0.57±0.046; with number of kernels in 25 c. c., 0.56 ±0.046; and with per cent of soft starch, −0.55±0.047]

Group	Range of expansion (volumes)	Number of ears	Average			
			Weight of 100 kernels (grams)	Number of kernels in 25 cubic centimeters	Percentage of soft starch	Expansion (volumes)
A	5–7	12	15.5	130	11.2	6.0
B	8–10	14	15.2	136	9.9	9.4
C	11–13	20	14.0	148	7.8	12.0
D	14–16	20	14.1	145	8.2	15.0
E	17–19	11	12.9	158	7.2	17.9
F	20–22	13	11.4	180	3.8	20.5
G	23–25	10	11.6	177	2.6	24.2

The relation of percentage of soft starch to poppability also is pronounced. Group A has an average soft-starch content of 11.2 per cent, whereas group G, with the largest popping expansion, has an average soft-starch content of only 2.6 per cent. There is a consistent decrease in the soft-starch content from group to group when the groups are arranged in ascending order on the basis of expansion.

The tendencies shown in these groupings suggest at once the probability of significant correlations between expansion and the three kernel characters measured. The correlation coefficients were calculated and are shown in Table 2. These coefficients are all about twelve times their probable errors and are of undoubted significance.

INFLUENCE OF KERNEL CHARACTERS ON POPPABILITY IN YELLOW PEARL POP CORN

In order to verify and amplify the results of the preliminary studies, a second experiment was planned, in which a larger number of ears of another variety was used, and the scope of the work was extended to include three additional kernel measurements. A sample of 394 ears of Yellow Pearl, which had uniform conditions for growth and curing, was used as the experimental material. The weight of 100 kernels, number of kernels in 25 cubic centimeters, percentage of soft starch, and expansion were determined as before. In an attempt to analyze further the relation between kernel size and expansion, the three additional measurements of length, breadth, and thickness of kernels were also taken.

TABLE 3.—*The means, standard deviations, and coefficients of variability of the various characters studied in a group of 394 ears of Yellow Pearl pop corn*

Symbol	Character	Mean	Standard deviation	Coefficient of variability
V	Expansion.....volumes	24.20±0.078	2.29±0.055	9.45±0.229
S	Percentage of soft starch.....grams	8.09± .139	4.09± .098	50.59±1.495
W	Weight of 100 kernels.....grams	15.05± .060	1.77± .043	11.78± .287
N	Number of kernels in 25 cubic centimeters	143.10± .574	16.90± .406	11.81± .288
L	Length of kernel.....millimeters	8.08± .015	.45± .011	5.52± .133
B	Breadth of kernel.....do	5.70± .011	.33± .008	5.72± .138
T	Thickness of kernel.....do	3.48± .009	.28± .007	8.01± .194

The ordinary statistical constants of the various measurements, together with the symbols used hereafter for the various characters, are shown in Table 3.

GROSS CORRELATIONS

The coefficients of correlation between expansion and each of the kernel characters and also those between the various combinations of kernel characters, are given in Table 4.

TABLE 4.—*Gross correlations between all possible combinations of the various characters studied in a sample of 394 ears of Yellow Pearl pop corn*

Character	Expansion (volumes) *V*	Percentage of soft starch *S*	Weight of 100 kernels (grams) *W*	Number of kernels in 25 cubic centimeters *N*
Expansion *V*		−0.59±0.022	−0.31±0.031	0.38±0.029
Percentage of soft starch *S*	−0.59±0.022		.42± .028	−.47± .026
Weight 100 kernels *W*	−.31± .031	.42± .028		−.84± .010
Number of kernels in 25 cubic centimeters *N*	.38± .029	−.47± .026	−.84± .010	
Length of kernels *L*	−.44± .028	.19± .033	.09± .034	−.12± .033
Breadth of kernels *B*	−.29± .031	.15± .033	.16± .033	−.18± .033
Thickness of kernels *T*	−.17± .033	.17± .033	.08± .034	−.13± .033

Character	Length of kernels (millimeters) *L*	Breadth of kernels (millimeters) *B*	Thickness of kernels (millimeters) *T*
Expansion *V*	−0.44±0.028	−0.29±0.031	−0.17±0.033
Percentage of soft starch *S*	.19± .033	.15± .033	.17± .033
Weight 100 kernels *W*	.09± .034	.16± .033	.08± .034
Number of kernels in 25 cubic centimeters *N*	−.12± .033	−.18± .033	−.13± .033
Length of kernels *L*		.19± .033	.07± .034
Breadth of kernels *B*	.19± .033		.09± .034
Thickness of kernels *T*	.07± .034	.09± .034	

The correlations between expansion and the first three kernel characters confirm the results of the preliminary study on White Rice pop corn, although the coefficients for weight of kernel and for number of kernels in a given volume are somewhat lower. The correlations involving expansion all show that within this sample those ears with the smaller kernels and those with kernels containing the least proportion of soft starch, in general, give the greatest increase in volume on popping. The percentage of soft starch in the endosperm had the greatest influence on popping yield of any of the kernel characters considered in this study, the coefficient of correlation in this case being −0.59 and 27 times its probable error. The conclusion that ears with the most horny and vitreous kernels tend to give the largest popping yields appears to be fully warranted.

The further analysis of the various relations shows that length, breadth, and thickness of kernel are all negatively correlated with the expansion. This would be expected, as these measurements largely determine the size of the kernel. The correlation of expansion is greatest with length of kernel and least with thickness of kernel. All of these correlation coefficients are statistically significant.

Considering the correlations of kernel characters among themselves, the greatest is between number of kernels per given volume and

weight of kernels and, of course, is negative. Interesting points brought out in Table 4 are the relatively high correlations between weight and starchiness (0.42 ± 0.028) and between size of kernels (as measured by number of kernels in 25 c. c.) and starchiness (-0.47 ± 0.026). These values indicate that large kernels are much more likely than small ones to have a large proportion of soft starch and suggest that one reason for the relatively poor popping quality of the ears with the larger kernels may be the associated higher percentage of soft starch which they contain.

The remaining correlations among the kernel characters are comparatively small. Those coefficients of 0.11 or more are over three times their probable errors and may be considered as probably significant. It is rather surprising that the correlations between weight of kernel and length, breadth, and thickness of kernel are not higher. Length and thickness are more highly correlated with starchiness than with weight, and breadth is correlated about equally with starchiness and with weight.

MULTIPLE CORRELATIONS

In the gross correlations considered in the previous paragraphs, the tendencies toward concomitant variation between poppability and certain individual kernel characters have been pointed out. These kernel characters, in turn, have been shown to be more or less highly correlated among themselves. In order to ascertain the relationships between expansion and the combined effect of all the other characters reported, or various groups of them, the method of multiple correlations was used. The coefficients of multiple correlations of expansion with various combinations of kernel characters are given in Table 5.

TABLE 5.—*Coefficients of multiple correlation between expansion and various combinations of kernel characters, ranked according to their numerical value*

[For explanation of symbols, see Table 3]

Characters	Coefficient (R)	Characters	Coefficient (R)	Characters	Coefficient (R)	Characters	Coefficient (R)
$V(SWNLBT)$	0.731	$V(SL)$	0.678	$V(SW)$	0.597	$V(WNBT)$	0..478
$V(SWNLB)$	.729	$V(SWNBT)$	.674	$V(WNLB)$	.589	$V(NBT)$	.458
$V(SNLBT)$	.718	$V(SWNB)$	.673	$V(NLBT)$	.587	$V(LT)$	.457
$V(SWNLT)$	.715	$V(SWNT)$	.644	$V(NLB)$	.587	$V(WNB)$	.445
$V(SWNL)$	.714	$V(SWN)$	.643	$V(WNLT)$	.578	$V(WNT)$	.417
$V(SNLB)$	.701	$V(SNBT)$	.635	$V(WNL)$	.564	$V(WBT)$	.416
$V(SWLBT)$	.697	$V(SNB)$	.633	$V(NLT)$	.561	$V(NB)$	.410
$V(SLBT)$	.696	$V(SWBT)$	.630	$V(NL)$	.557	$V(NT)$	.402
$V(SWLB)$	.695	$V(SBT)$	.629	$V(WLBT)$	.553	$V(WB)$	.396
$V(SLB)$	.694	$V(SWB)$	.628	$V(NL)$	.548	$V(WN)$	.355
$V(SNLT)$	.688	$V(SB)$	.626	$V(WLB)$	.541	$V(WT)$	.344
$V(SNL)$	.686	$V(SNT)$	.608	$V(WLT)$	.528	$V(BT)$	.325
$V(SWLT)$	.684	$V(SN)$	.604	$V(WL)$	.513		
$V(SWL)$	.681	$V(SWT)$	.601	$V(LBT)$	.499		
$V(SLT)$	.681	$V(ST)$	.597	$V(LB)$	.482		

When all six kernel characters are considered with expansion, $R=0.731$. This value for R may be substituted in the formula $100\ (1-\sqrt{1-R^2})$ to find the proportion of variability in expansion directly associated with the kernel characters studied. The value obtained, 31.8 per cent, indicates that approximately one-third of this variability is accounted for by variations having to do with size

and starchiness of the unpopped kernels. Other factors, such, perhaps, as the size of the starch grains or the tenacity or elasticity of the surrounding matrix, may play an important part in determining the degree of expansion.

In the sample studied, however, fairly reliable predictions of poppability could be made on the basis of the size and soft-starch content of the kernels, two easily observable characters.

The importance of the influence of starchiness on popping quality may be noted from the fact that the correlation between expansion and percentage of soft starch ($r_{vs} = -0.593$, Table 4) is slightly greater than the multiple correlation between expansion and all the kernel characters studied other than starchiness ($R_{v(WNLBT)} = 0.589$, Table 5).

PARTIAL CORRELATIONS

The partial correlation method is especially valuable for purposes of analysis in a study of this kind, in which several related characters are more or less highly correlated among themselves.

The various partial correlations resulting from the study are given in Table 6. In each case the first two letters indicate the variables for which the correlation is calculated with the characters indicated by the subsequent letters held constant. The coefficients, therefore, measure the correlation between expansion and one of the kernel characters, the influence of various combinations of the other characters being eliminated. The coefficients for any two characters are arranged in order of their magnitude.

The partial correlation coefficients for expansion and percentage of soft starch are high throughout, irrespective of what combinations of other characters are held constant. Of all the kernel characters studied, the proportion of soft starch seems to be most closely associated with popping expansion and to be most nearly free from influence by the other characters studied.

The partial correlations of expansion with weight of kernel present an interesting situation. The coefficients are all negative when the number of kernels is variable, but all positive in those combinations in which number of kernels is held constant. In other words, the popping expansion is highest in general in the lightest kernels, but in the case of kernels of a given size the popping expansion is slightly greater in the heavier ones. This suggests at once that probably there is a positive relation between popping expansion and specific gravity. The fact that pop corn has a considerably higher test weight per measured bushel than does field corn lends support to this hypothesis. No determinations of kernel density were made on the ears studied.

The partial coefficients for expansion with number of kernels per 25 cubic centimeters are all positive. There is a decided drop in those coefficients, however, in which starchiness is held constant. The smaller coefficients in every instance are combinations which contain starchiness as one of the constant factors.

The correlations of expansion with length of kernel are negative and are all of undoubted significance, the smallest coefficient being thirteen times its probable error. These coefficients differ little, irrespective of what characters are held constant.

TABLE 6.—*Partial correlations of expansion with each kernel character studied when various combinations of the other characters are held constant*

[For explanation of symbols see Table 3]

Characters	r	Characters	r	Characters	r	Characters	r
VS. T	−0.58	*VW. NT*	0.01	*VL. T*	−0.43	*VB. WL*	−0.20
VS. B	−.58	*VW. NLBT*	.01	*VL. W*	−.43	*VB. SL*	−.20
VS. L	−.58	*VW. NL*	.01	*VL. WT*	−.43	*VB. SLT*	−.20
VS. LB	−.57	*VW. NBT*	.02	*VL. N*	−.42	*VB. WLT*	−.19
VS. BT	−.57	*VW. N*	.02	*VL. WN*	−.42	*VB. SWL*	−.19
VS. LT	−.57	*VW. NLB*	.02	*VL. NT*	−.42	*VB. WNL*	−.19
VS. LBT	−.56	*VW. NB*	.03	*VL. WNT*	−.42	*VB. NL*	−.19
VS. W	−.54	*VW. NSLT*	.04	*VL. SW*	−.41	*VB. SWLT*	−.19
VS. WB	−.53	*VW. NSL*	.05	*VL. S*	−.41	*VB. SWNL*	−.19
VS. WT	−.52	*VW. NST*	.05	*VL. SN*	−.41	*VB. WNLT*	−.19
VS. WL	−.52	*VW. NSLBT*	.05	*VL. SWT*	−.41	*VB. NLT*	−.19
VS. WBT	−.52	*VW. NSLB*	.05	*VL. SWN*	−.41	*VB. SWNLT*	−.19
VS. WLB	−.52	*VW. NS*	.05	*VL. ST*	−.41	*VB. SNLT*	−.18
VS. WLT	−.51	*VW. NSBT*	.05	*VL. SNT*	−.41	*VB. SNL*	−.18
VS. WLBT	−.51	*VW. NSB*	.06	*VL. SWNT*	−.41	*VT. L*	−.16
VS. NW	−.51	*VN. L*	.37	*VL. B*	−.40	*VT. W*	−.16
VS. N	−.51	*VN. T*	.37	*VL. WB*	−.40	*VT. B*	−.15
VS. NWB	−.50	*VN. LT*	.36	*VL. BT*	−.40	*VT. LB*	−.15
VS. NB	−.50	*VN. B*	.35	*VL. WBT*	−.40	*VT. WL*	−.14
VS. NWT	−.50	*VN. LB*	.35	*VL. NB*	−.40	*VT. WB*	−.14
VS. NT	−.50	*VN. BT*	.34	*VL. WNB*	−.40	*VT. WLB*	−.13
VS. NWBT	−.50	*VN. LBT*	.34	*VL. NBT*	−.40	*VT. N*	−.13
VS. NWL	−.50	*VN. W*	.24	*VL. WNBT*	−.40	*VT. WN*	−.13
VS. NBT	−.50	*VN. WL*	.22	*VL. SWB*	−.39	*VT. NL*	−.12
VS. NWLB	−.49	*VN. WB*	.22	*VL. SWNB*	−.38	*VT. WNL*	−.12
VS. NL	−.49	*VN. WT*	.22	*VL. SNBT*	−.38	*VT. NB*	−.12
VS. NLB	−.49	*VN. WLB*	.22	*VL. SWBT*	−.38	*VT. WNB*	−.12
VS. NWLT	−.49	*VN. WLT*	.21	*VL. SB*	−.38	*VT. NLB*	−.11
VS. NWLBT	−.49	*VN. WBT*	.21	*VL. SWNBT*	−.38	*VT. WNLB*	−.11
VS. NLT	−.49	*VN. WLBT*	.20	*VL. SBT*	−.38	*VT. S*	−.09
VS. NLBT	−.49	*VN. S*	.15	*VB. T*	−.28	*VT. SW*	−.09
VW. T	−.30	*VN. SL*	.14	*VB. W*	−.26	*VT. SL*	−.08
VW. L	−.30	*VN. ST*	.14	*VB. S*	−.25	*VT. SN*	−.08
VW. LT	−.30	*VN. SLT*	.14	*VB. WT*	−.25	*VT. SWL*	−.08
VW. B	−.28	*VN. SW*	.13	*VB. WN*	−.25	*VT. SWN*	−.08
VW. LB	−.28	*VN. SWL*	.12	*VB. ST*	−.25	*VT. SWB*	−.08
VW. BT	−.27	*VN. SWT*	.12	*VB. N*	−.25	*VT. SB*	−.08
VW. LBT	−.27	*VN. SLB*	.12	*VB. SW*	−.24	*VT. SNL*	−.07
VW. SL	−.09	*VN. SLBT*	.12	*VB. WNT*	−.24	*VT. SWLB*	−.07
VW. SLT	−.09	*VN. SB*	.12	*VB. NT*	−.24	*VT. SLB*	−.07
VW. S	−.09	*VN. SWB*	.12	*VB. SWT*	−.24	*VT. SWNL*	−.07
VW. ST	−.09	*VN. SWLT*	.12	*VB. SWN*	−.24	*VT. SNB*	−.07
VW. SLBT	−.07	*VN. SBT*	.12	*VB. SN*	−.24	*VT. SWLB*	−.07
VW. SLB	−.07	*VN. SWLB*	.12	*VB. SWNT*	−.23	*VT. SNLB*	−.07
VW. SBT	−.06	*VN. SWBT*	.11	*VB. SNT*	−.23	*VT. SWNLB*	−.06
VW. SB	−.06	*VN. SWLBT*	.11	*VB. L*	−.23		
VW. NLT	.01	*VL. SNB*	−.43	*VB. LT*	−.22		

Correlations of 0.11 are more than three times their probable errors, those of 0.14 more than four times, those of 0.17 more than five times, those of 0.31 more than ten times, those of 0.43 more than fifteen times, and correlations of more than 0.51 are more than twenty times their probable errors.

The correlations between expansion and breadth of kernel and between expansion and thickness of kernel are all small, particularly those of the latter group.

DISCUSSION

Of the characters studied, the proportion of soft starch seems to be the outstanding determining influence. Sectioning kernels from each individual ear is rather tedious, and it requires experience to estimate with accuracy the relative amounts of soft starch. The high correlation between starchiness and size of kernel, however, may serve as a rough guide to eliminate many of the ears having the softest kernels. Growers frequently have a tendency to select large seed ears, which usually have kernels above the average in size and starchiness and may represent a more or less distant contamination with field corn. This practice should be avoided if quality is to be maintained.

The fact that the results of the two experiments here reported, made with samples from different varieties, confirm each other so closely lends support to the view that the relationships are of a general nature and are not limited to the varieties here considered. It should not be overlooked, however, that although some of the correlations are high the multiple correlation between expansion and all the kernel characters combined was only 0.731. The associations between kernel characters and popping expansion should be a valuable aid in selection of pop corn for seed, but they can not entirely replace a popping test of prospective seed ears as a means of crop improvement.

SUMMARY

In pop corn a moisture content of about 12 per cent is optimum for greatest expansion in popping.

Within a given variety, those ears bearing kernels with the least soft starch are likely, on the average, to give the largest expansion in popping.

Similarly, those ears having smaller kernels are more likely to give a higher expansion on popping than those with larger kernels.

Study of these kernel characters should be a valuable aid in selecting seed to maintain and improve the popping quality of a strain of pop corn, although it can not wholly supplant an actual popping test of individual ears.

THE RELATIVE ASSIMILATION BY DAIRY COWS OF CLOVER AND ALFALFA HAYS AND OF RATIONS OF DIFFERENT CALCIUM AND PHOSPHORUS CONTENT[1]

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INTRODUCTION

The rôle of the Ca/P ratio in the diet as a cause of rickets or osteoporosis in rats has been the subject of considerable investigation. Park (*6*)[2] has reviewed the literature bearing on this point. It has been shown that excessive amounts of either calcium or phosphorus in the ration will produce rickets. When both are present in about equal and sufficient quantities and the other components of the ration are satisfactory, rickets does not develop.

It has seemed likely that an unbalancing of the Ca/P ratio in the rations of dairy cows might lead to poor assimilation of calcium and phosphorus and a condition of osteoporosis, as indicated by negative balances. In the usual feeding practice no thought is given to the relative amounts of calcium and phosphorus in the feeds. Although the calcium and phosphorus contents of feeds vary somewhat, it is possible that some attention given to this point may result in improved animal nutrition. It was with the idea of investigating this possibility that the present work was undertaken, it being thought that the investigation of the assimilability of the calcium and phosphorus of alfalfa and clover hays would be both interesting and profitable.

COWS USED

The experiments were conducted in an attempt to learn whether there is a difference in the degree of assimilability by dairy cows of (1) the calcium and phosphorus of clover and alfalfa hays and (2) the calcium and phosphorus in diets in which the ratio Ca/P is varied. Three cows were used for the work. Cow 253, a purebred Holstein, was used in both experiments. The other cows, 412 and 441, were purebred Jerseys. Cow 412 was to have been used throughout both experiments; but since much difficulty was experienced in inducing her to consume her ration, she was replaced in the second experiment by cow 441. The cows were approximately 7 years old and had just finished a year's official test for milk production about four months previous to the beginning of the experiment. In the interim all the cows had calved and were therefore at their highest plane of milk production. They had had no pasture or green feed for more than a year before the experiment was begun.

[1] Received for publication July 14, 1927; issued November, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 635.

FEEDS USED

During the first experiment the cows received grain mixture 75, consisting of 40 kgm. wheat bran, 40 kgm. corn meal, 20 kgm. linseed-oil meal, and 1 kgm. salt. In addition to the grain, they received as roughage during the first three weeks a well-cured clover hay grown on the dairy experiment farm at Beltsville, Md. The clover was cut May 26, 1925, in the early stage of maturity and was fed in March, 1926. During the latter half of the first experiment alfalfa hay was substituted for clover. This alfalfa hay was a baled product received from Kansas City on March 30, 1925, and fed in April, 1926. It had therefore been at the farm for more than a year before it was used, and as it was received at Beltsville in early spring it was probably cut six to nine months before it was shipped. It appeared to be of good quality, but nothing is known regarding its curing. Both hays were chopped and sacked before use.

During the second experiment the cows received grain mixture 160, a feed of low phosphorus content, consisting of 10 kgm. corn meal, 20 kgm. corn gluten feed, 40 kgm. linseed-oil meal, 30 kgm. ground oats, and 1 kgm. salt. The same lot of alfalfa hay used in the first experiment was fed throughout the second experiment. During the last three weeks of the second experiment sodium phosphate was added to the grain in sufficient amount to obtain a value of about 1.25 for the Ca/P ratio in the ration. Monosodium phosphate was fed May 26 to 31 and June 4 to 8, and disodium phosphate was fed the remainder of the time.

EXPERIMENTAL METHODS

With the exception of the phosphorus determination, the methods of analysis used in these experiments were the same as those employed in previous work in this laboratory (*3*, *4*, *5*). Instead of the tedious gravimetric method for phosphorus used in previous experiments at Beltsville, the colorimetric method, as recently modified by Roe, Irish, and Boyd (*7*), was employed. However when a pure sodium phosphate solution was used as a standard for comparison, it was not found possible to obtain correct results by this method, since the different concentrations of salts and acids in the various materials analyzed apparently affected the depth of color obtained. For each particular type of material to be analyzed, therefore (feces, milk, grain, or hay), a solution of a sample of that material on which a gravimetric determination had already been made, was used as the standard for colorimetric comparison of the other samples of the same type. This was found to give very satisfactory results and to effect a considerable saving of time.

The details of the experimental routine were much the same as those described in a former publication of this bureau (*5*). The feces and urine were collected automatically. The samples were preserved in the same manner as formerly. The feeding and the collection periods were computed in the same manner; that is, the feeding periods preceded by two days the collection periods in order to compensate for the lag in elimination of excreta. Tap water was furnished the cows by automatic drinking cups, which were filled from individual storage tanks over each stall. The daily intake of water was

recorded, the average of which varied from 47 liters in March for cow 412 to 80 liters in June for cow 253. As 1 liter of tap water was found to contain only 2 mgm. of calcium the effect of the water on the total calcium intake was regarded as insignificant. Throughout the experiments the cows were confined in a barn without exposure to sunlight. A self-recording thermometer gave information in regard to temperature, the variation of which was from 44° to 85° F. during the experiments. The daily rations of hay and grain were given about 2 p. m. and 5 a. m.

EXPERIMENTAL DATA

The percentage composition of the feeds used throughout the experiments is shown in Table 1. The amounts of feeds consumed are given in Table 2 and the percentage composition of the milk in Table 3.

TABLE 1.—*Composition of feeds as fed in both experiments*

Period	Feed	H_2O	Ca	P	N
		Per cent	*Per cent*	*Per cent*	*Per cent*
Mar. 17–Apr. 6	Clover hay	12.70	1.384	0.153	1.903
Apr. 7–May 4	Alfalfa hay (I)	12.04	1.532	.172	2.654
May 5–June 13	do	12.86	1.518	.171	2.629
June 14–15	Alfalfa hay (II)	9.93	1.275	.237	2.721
Mar. 17–Apr. 6	Grain 75 (I)	12.71	.0995	.745	2.574
Apr. 7–22	do	12.23	.100	.749	2.588
Apr. 23–May 4	Grain 75 (II)	12.93	.0805	.535	2.273
May 5–25	Grain 160	9.74	.308	.524	3.812
May 26–June 15	do	10.12	.306	.522	3.796

TABLE 2.—*Feed records of the cows used in both experiments*

Week	Cow 253			Cow 412		
	Grain offered	Hay offered	Feed refused	Grain offered	Hay offered	Feed refused
	Kgm.	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*
Mar. 17–23	56.0	42.0	0.5 hay	55.9	30	8.16 hay and grain.
Mar. 24–30	60.2	46.2	0.4 hay	45.5	34	0.8 hay.
Mar. 31–Apr. 6	63.0	49.0	3.6 hay and grain	53.2	35	0.6 hay.
Apr. 7–13	57.6	53.2	None	49.4	30	None.
Apr. 14–20	60.2	53.2	do	51.2	35	Do.
Apr. 21–27	59.2	53.2	do	34.2	35	Do.
Apr. 28–May 4	60.2	53.2	do	41.4	35	Do.
				Cow 441		
May 5–11	55.9	53.2	None	56	54.5	None.
May 12–18	60.2	53.2	do	56	56	Do.
May 19–25	60.2	56.0	do	56	56	Do.
May 26–June 1	56.0	56.0	do	56	56	Do.
June 2–8	56.0	56.0	do	56	55	Do.
June 9–15	56.0	56.0	do	56	56	Do.

TABLE 3.—*Average daily composition of milk from cows used in both experiments*

Week	Cow 253			Cow 412		
	Ca	P	N	Ca	P	N
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Mar. 19–25	0.105	0.091	0.475	0.145	0.119	0.603
Mar. 26–Apr. 1	.104	.089	.454	.146	.119	.601
Apr. 2–8	.106	.088	.435	.141	.119	.602
Apr. 9–15	.101	.091	.468	.140	.114	.584
Apr. 16–22	.101	.090	.457	.142	.117	.620
Apr. 23–29	.100	.092	.457	.138	.115	.595
Apr. 30–May 6	.098	.093	.476	.141	.118	.606
				Cow 441		
May 7–13	.093	.092	.474	0.148	0.123	0.657
May 14–20	.096	.091	.481	.147	.124	.666
May 21–27	.096	.091	.485	.145	.120	.662
May 28–June 3	.097	.092	.484	.145	.119	.666
June 4–10	.097	.095	.481	.148	.123	.691
June 11–17	.097	.093	.483	.145	.122	.684

On March 12, 1926, cows 253 and 412 were placed in metabolism stalls. Cow 253 weighed 513 kgm. at the beginning of the experiment and 507 kgm. at the end. Cow 412 weighed 481 kgm. at the beginning of the experiment and 426 kgm. at the end. The experiment terminated on May 6. The records of balances and assimilations are presented in Tables 4 and 5.

The second experiment followed immediately. In anticipation of replacing cow 412 by cow 441, the latter was placed in a metabolism stall on May 5, and both cow 253 and cow 441 were given the ration which they were to receive in the second experiment. Cow 253 weighed 507 kgm. at the beginning of the experiment and 501 kgm. at the end. Cow 441 weighed 518 kgm. at the beginning of the experiment and 517 kgm. at the end. The experiment terminated on June 17. The results of the experiment are given in Tables 6 and 7.

TABLE 4.—*Daily calcium, phosphorus, and nitrogen balances and assimilations and milk yield of cow 253, March 19 to May 6, 1926*

Week	Ca in urine and feces	Ca in milk	Total Ca excreted	Ca intake	Ca balance	Ca assimilation		Milk yield	P in urine and feces	P in milk	Total P excreted	P intake	P balance	P assimilation		N in urine and feces	N in milk	Total N excreted	N intake	N balance	N assimilation	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*	*Kgm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*
CLOVER PERIOD																						
Mar. 19–25	82.5	19.5	102.0	90.9	−11.1	8.4	9.2	18.6	55.0	16.9	71.9	68.8	−3.1	13.8	20.0	213.4	88.3	301.7	318.7	+17.0	105.3	33.0
Mar. 26–Apr. 1	91.4	20.0	111.4	99.8	−11.6	8.4	8.4	19.2	60.3	17.1	77.4	74.2	−3.3	13.8	18.6	248.1	87.3	335.4	345.9	+10.5	97.8	28.3
Apr. 2–8	98.9	20.5	119.4	103.8	−15.6	4.9	4.7	19.4	59.8	17.0	76.8	75.3	−1.6	15.4	20.5	263.2	84.2	347.4	354.3	+6.9	91.1	25.7
Average	90.9	20.0	110.9	98.2	−12.8	7.2	7.4	19.1	58.4	17.0	75.4	72.8	−2.7	14.3	19.7	241.6	86.6	328.2	339.6	+11.5	98.1	29.0
ALFALFA PERIOD																						
Apr. 9–15	108.5	20.5	129.0	124.7	−4.3	16.1	12.9	20.3	62.7	18.4	81.1	74.7	−6.4	12.0	16.6	306.9	94.9	401.8	414.7	+12.9	107.8	26.0
Apr. 16–22	110.0	20.0	130.0	125.0	−5.0	15.1	12.1	19.8	60.5	17.9	78.4	77.5	−0.9	17.0	21.9	318.8	90.7	409.5	424.3	+14.7	105.4	24.9
Apr. 23–29	111.6	20.3	131.9	123.7	−8.2	12.1	9.8	20.3	44.8	18.7	63.5	63.6	+0.1	18.8	29.6	290.7	92.9	383.6	401.7	+18.1	111.0	27.6
Apr. 30–May 6	110.5	19.7	130.2	123.4	−6.8	12.9	10.4	20.1	42.7	18.7	61.4	59.1	−2.4	16.3	27.7	306.4	95.7	402.1	397.2	−4.9	90.8	22.9
Average	110.2	20.1	130.3	124.2	−6.1	14.1	11.3	20.1	52.7	18.4	71.1	68.7	−2.4	16.0	24.0	305.7	93.6	399.3	409.5	+10.2	103.8	25.4

TABLE 5.—*Daily calcium, phosphorus, and nitrogen balances and assimilations and milk yield of cow 412, March 19 to May 6, 1926*

Week	Ca in urine and feces	Ca in milk	Total Ca excreted	Ca intake	Ca balance	Ca assimilation		Milk yield	P in urine and feces	P in milk	Total P excreted	P intake	P balance	P assimilation		N in urine and feces	N in milk	Total N excreted	N intake	N balance	N assimilation	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*	*Kgm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*
CLOVER PERIOD																						
Mar. 19–25	64.1	17.2	81.3	77.4	−3.9	13.3	17.1	11.8	47.8	14.1	61.9	63.1	+1.2	15.3	24.2	192.7	71.4	264.1	288.0	+23.9	95.3	33.1
Mar. 26–Apr. 1	71.1	17.2	88.3	73.5	−14.8	2.5	3.4	11.8	50.6	14.0	64.6	55.8	−8.8	5.3	9.4	204.0	70.9	274.9	257.6	−17.4	53.6	20.8
Apr. 2–8	67.0	17.8	84.8	76.6	−8.2	9.6	12.5	12.6	48.9	15.0	63.9	64.3	+0.4	15.4	24.0	219.7	76.0	295.7	288.9	−6.8	69.2	24.0
Average	67.4	17.4	84.8	75.8	−9.0	8.5	11.0	12.1	49.1	14.4	63.5	61.1	−2.4	12.0	19.2	205.5	72.8	278.2	278.2	−0.1	72.7	26.0
ALFALFA PERIOD																						
Apr. 9–15	67.7	15.4	83.1	72.7	−10.4	5.0	6.9	11.0	53.1	12.5	65.6	60.2	−5.4	7.1	11.8	232.3	64.2	296.5	296.4	−0.1	64.1	21.6
Apr. 16–22	62.4	16.6	79.0	83.9	+4.9	21.5	25.6	11.7	43.0	13.7	56.7	63.4	+6.7	20.3	32.1	230.6	72.5	303.1	322.0	+18.9	91.3	28.4
Apr. 23–29	70.8	14.3	85.1	81.0	−4.1	10.2	12.6	10.4	29.6	11.9	41.5	39.4	−2.1	9.8	24.9	181.2	61.6	242.8	250.6	+7.7	69.3	27.7
Apr. 30–May 6	70.3	15.6	85.9	81.4	−4.5	11.0	13.6	11.0	26.5	13.0	39.5	40.2	+0.7	13.7	34.1	191.3	66.8	258.1	267.1	+9.0	75.8	28.4
Average	67.8	15.5	83.3	79.8	−3.5	11.9	14.7	11.0	38.1	12.8	50.8	50.8	0.0	12.7	25.7	208.9	66.3	275.1	284.0	+8.9	75.1	26.5

TABLE 6.—*Daily calcium, phosphorus, and nitrogen balances, assimilations, milk yield, and Ca/P intake of cow 253, May 7 to June 17, 1926*

Week	Ca in urine and feces	Ca in milk	Total Ca excreted	Ca intake	Ca balance	Ca assimilation		Ca/P intake	Milk yield	P in urine and feces	P in milk	Total P excreted	P intake	P balance	P assimilation		N in urine and feces	N in milk	Total N excreted	N intake	N balance	N assimilation	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*		*Kgm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Pr. ct. of intake*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*
LOW PHOSPHORUS PERIOD																							
May 7–13	126.2	18.2	144.4	140.0	−4.4	13.7	9.8	2.55	19.5	43.6	18.0	61.6	54.8	−6.8	11.2	20.5	376.4	92.6	469.0	504.2	+35.2	127.8	25.3
May 14–20	123.0	18.5	141.5	141.9	+0.4	18.9	13.3	2.44	19.3	42.6	17.6	60.2	58.1	−2.1	15.5	26.7	387.2	92.8	480.0	527.6	+47.6	140.4	26.6
May 21–27	129.5	18.3	147.8	147.9	+0.1	18.4	12.5	2.52	19.1	51.0	17.4	68.4	58.7	−9.6	7.8	13.2	404.9	92.7	497.6	538.1	+40.6	133.3	24.8
Average	126.2	18.3	144.6	143.3	−1.3	17.0	11.9	2.50	19.3	45.7	17.7	63.4	57.2	−6.2	11.5	20.1	389.5	92.7	482.2	523.3	+41.1	133.8	25.6
HIGH PHOSPHORUS PERIOD																							
May 28–June 3	116.2	18.4	134.6	145.9	+11.3	29.7	20.4	1.10	19.0	99.2	17.5	116.7	132.9	+16.2	33.7	25.4	269.5	91.9	461.4	514.0	+52.6	144.5	28.1
June 4–10	120.7	18.1	138.8	145.9	+7.1	25.2	17.3	1.39	18.7	82.1	17.8	99.9	104.6	+4.7	22.5	21.5	384.4	89.9	474.3	514.0	+39.7	129.6	25.2
June 11–17	120.4	17.7	138.1	140.4	+2.2	20.0	14.2	1.28	18.3	90.8	17.0	107.8	109.6	+1.8	18.7	17.1	394.8	88.2	483.0	516.1	+33.1	121.3	23.5
Average	119.1	18.1	137.2	144.1	+6.9	25.0	17.3	1.26	18.7	90.7	17.4	108.1	115.7	+7.6	25.0	21.3	382.9	90.0	472.9	514.7	+41.8	131.8	25.6

TABLE 7.—*Daily calcium, phosphorus, and nitrogen balances, assimilations, milk yield, and Ca/P intake of cow 441, May 7 to June 17, 1926*

LOW PHOSPHORUS PERIOD

Week	Ca in urine and feces	Ca in milk	Total Ca excreted	Ca intake	Ca balance	Ca assimilation		Ca/P intake	Milk yield	P in urine and feces	P in milk	Total P excreted	P intake	P balance	P assimilation		N in urine and feces	N in milk	Total N excreted	N intake	N balance	N assimilation	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*		*Kg. m*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*P. ct. of intake*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent of intake*
May 7–13	117.3	19.3	136.6	142.8	+6.2	25.5	17.9	2.59	13.1	40.1	16.1	56.2	55.2	−0.9	15.2	27.5	373.6	85.9	459.5	509.6	+50.2	136.1	26.7
May 14–20	124.3	19.2	143.5	146.1	+2.6	21.7	14.9	2.63	13.0	39.1	16.2	55.3	55.6	+0.3	16.5	29.7	388.1	86.9	475.0	515.3	+40.3	127.2	24.7
May 21–27	132.5	*18.1	150.6	146.1	−4.5	13.6	9.3	2.63	12.5	55.0	*15.0	70.0	55.6	−14.4	.6	1.1	405.9	*82.7	488.6	515.3	+26.7	109.4	21.2
Average	124.7	18.9	143.6	145.0	+1.4	20.3	14.0	2.62	12.9	44.7	15.8	60.5	55.5	−5.0	10.8	19.4	389.2	85.2	474.4	513.4	+39.1	124.2	24.2

HIGH PHOSPHORUS PERIOD

May 28–June 3	124.0	17.8	141.8	145.9	+4.2	21.9	15.0	1.10	12.3	104.4	14.6	119.0	132.9	+13.9	28.4	21.4	401.8	81.6	483.4	514.0	+30.6	112.2	21.8
June 4–10	123.6	17.5	141.1	143.8	+2.6	20.1	14.0	1.38	11.9	81.8	14.6	96.4	104.4	+8.0	22.6	21.7	403.7	81.9	485.6	510.2	+24.6	106.5	20.9
June 11–17	129.2	17.0	146.2	140.4	−5.8	11.2	8.0	1.28	11.7	92.8	14.3	107.1	109.6	+2.5	16.7	15.3	409.8	80.0	489.8	516.1	+26.3	106.3	20.6
Average	125.6	17.4	143.0	143.4	+0.3	17.7	12.3	1.25	12.0	93.0	14.5	107.5	115.6	+8.1	22.6	19.5	405.1	81.2	486.3	513.4	+27.2	108.3	21.1

• Estimated from data for 6 days only.

By using a method adopted in a former paper (*5*), the body losses and gains of calcium and phosphorus for each cow are shown graphically in Figure 1. Each cow is assumed to have 7,000 gm. of calcium and 3,255 gm. of phosphorus in her bones at the beginning of the experiment. The record of cow 253 is shown as a continuous graph throughout both experiments, whereas the graphs for the other animals are shown only for the experiment in which they were used. It is apparent that in the beginning of the clover and alfalfa experiment both cows showed a fairly rapid loss of both calcium and phosphorus, followed by a slower steady loss. In the low and high phosphorus

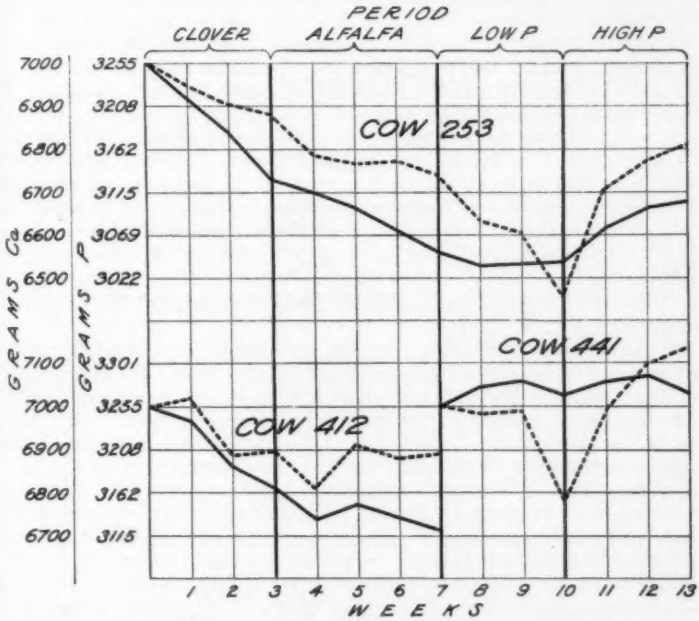

FIG. 1.—Curves showing the losses and gains of calcium and phosphorus by cows during the experiments. The solid line represents the change in the calcium content in the body; the broken line the change in the phosphorus content

experiment the results were quite different. The losses in the beginning were followed by gains, and at the end of the experiment both animals had more calcium and phosphorus in their bodies than at the beginning.

DISCUSSION

Cow 253 shows negative calcium and phosphorus balances through practically all the first experiment. The negativity of the calcium balance is reduced more than one-half, however, by substituting alfalfa for clover. The assimilation also shows a very decided improvement in the alfalfa period. The differences in the phosphorus balances and assimilations are not so marked, although they show a tendency in the same direction. These improved balances and

assimilations are accompanied by an increased milk yield. The nitrogen balances show that the cow suffered no digestive disturbance. Cow 412 likewise showed negative calcium and phosphorus balances through the greater part of the experiment. There was a general improvement in the character of her balances and assimilations in the alfalfa period, although her milk yield dropped somewhat. Considerable difficulty was encountered in inducing her to consume an adequate ration. The character of her nitrogen balances, three of which were negative, shows a somewhat disturbed digestive condition. Both cows assimilated a greater proportion of calcium during the alfalfa period in spite of the fact that the calcium intake was greater.

It would appear from this experiment that calcium is better assimulated by dairy cows in the form of alfalfa than in the form of clover. Apparently both hays were of good quality although the alfalfa when fed was probably a year older than the clover. It is not known what factor facilitates the assimilation of calcium from alfalfa. Whether it is the fat-soluble vitamin or some other unsuspected factor is a matter of speculation. In a recent publication from this laboratory it was shown that alfalfa contains citric, malic, and malonic acids (*8*). Possibly the presence of these acids exerts a favorable effect on the assimilability of calcium. The expressed juice of green alfalfa has a P_H of 5.28.[3] It has been reported that a rickets-producing diet can be changed into a nonrickets-producing diet by increasing the acidity (*6*).

In the second experiment, where the phosphorus content of the ration was varied, cow 253, except during one week, showed positive calcium balances throughout. Calcium balances and assimilations were decidedly better in the high phosphorus period. The phosphorus balances were negative in the low phosphorus period and positive in the high phosphorus period. The phosphorus assimilation was larger also in the high phosphorus period and the percentage assimilation, even with a doubled phosphorus intake, remained constant. The nitrogen record indicates that the cow's digestion was normal. The performance of cow 441 was less noteworthy. Her calcium balances and assimilations were slightly poorer on the high phosphorus ration. Her phosphorus balances and assimilations were better, however, in the high phosphorus period, and the percentage assimilations, with a doubled phosphorus intake, remained constant. The nitrogen record shows that her digestion was normal.

Apparently cows can assimilate calcium and phosphorus when the Ca/P ratio in the feed is as high as 2.5, but a more favorable value for this ratio is probably from 1.0 to 1.5.

It is worthy of note that in this second experiment positive calcium and phosphorus balances have been obtained with a cow receiving a ration of alfalfa hay and a good grain mixture and yielding 19 kgm. of milk daily. So far as the authors know, such a performance has not previously been noted for a cow on so simple a ration. Hart (*1*) found that a cow could be kept in positive calicum balance while on a ration of good alfalfa hay, corn silage, and grain, and yielding about 19 kgm. of milk. He (*1*, *2*) also succeeded in obtaining positive calcium balances with cows on rations containing green feed. But the

[3] Unpublished data obtained at this laboratory.

great majority of calcium balances which have been determined in cows giving large amounts of milk on winter feeds have been negative.

The writers' observations on balance experiments lead them to believe that three conditions are important for insuring equilibrium or a positive balance in dairy cows in respect to calcium and phosphorus.

1. A sufficient quantity of calcium and phosphorus must be supplied in the ration.

2. The calcium and phosphorus should be supplied in about equal quantities. The amount of calcium probably should not exceed the amount of phosphorus by more than 50 per cent.

3. The calcium should be supplied as a constituent of natural feeds in a ration including a good quality of legume hay, preferably alfalfa, and a good grain mixture.

SUMMARY

Metabolism experiments with two lactating cows show a better assimilation of calcium and phosphorus from alfalfa hay than from clover hay.

Metabolism experiments involving the use of rations where the content of phosphorus was increased by the addition of sodium phosphate suggest a better assimilation of calcium and phosphorus when the value of the Ca/P ratio of the feed is 1.25 than when it is 2.5.

Positive calcium and phosphorus balances have been observed in the case of a cow on a ration of good alfalfa hay and a good grain mixture and yielding 19 kgm. of milk.

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THE EFFECT OF INANITION UPON THE YIELD AND COMPOSITION OF COWS' MILK[1]

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INTRODUCTION

Very little work has been reported on the effect of inanition upon the yield and composition of milk. Only one record was found in the literature of an inanition period as long as 48 hours. Several investigators report upon the effects of underfeeding. Lami (*2*)[2] starved a cow for 36 hours and at the end of the starvation period found increases in the percentages of total solids (13.6 to 14.3 per cent) and in nitrogenous matter and ash (4.2 to 6.25 per cent.) He found a slight reduction in fat (4.4 to 4.15 per cent) and a decided reduction in lactose (5 to 3.9 per cent).

Eckles and Palmer (*1*) studied the influence of underfeeding upon the milk of a number of cows. They found that high fat tests uniformly accompanied underfeeding, that there was no uniform effect on the percentages of the other milk components, and that the percentage of fat was not affected in any of the experiments.

Porcher (*4*) states that starvation for two days showed no appreciable injury to the milk.

Ragsdale and Swett (*5*) showed that a sudden reduction of the total amount of feed to one-half the normal amount increased the percentage of fat and decreased the amount of milk produced.

Taylor and Husband (*6*), in their study of the effect of variations in the nature of the diet upon the percentage composition of the milk of the goat, state that (1) the percentages of protein, fat, and ash vary inversely and the percentage of lactose varies directly as the daily volume, the greatest variation being shown by the fat and the least by the inorganic elements; (2) there is an inverse relationship between the percentage of lactose and the percentages of all the other constituents of the milk, this being particularly apparent in the case of the fat. They suggest that the quantity of lactose elaborated by the mammary glands controls the daily volume of the milk, and that, therefore, the rate of its elaboration controls the rate of milk secretion.

The present study was undertaken to learn the effect of total absence of feed upon the yield and the composition of the milk produced.

DESCRIPTION OF ANIMALS USED

Three cows, Nos. 1, 11, and 742, were used in this investigation. These cows, while in the experimental herd of the dairy department at the University of Illinois, gave a positive reaction to the tuberculin test and were isolated from the herd until slaughtered.

[1] Received for publication May 31, 1927; issued November, 1927. Presented before the division of biological chemistry at the seventy-third meeting of the American Chemical Society, Richmond, Va., Apr. 11 to 16, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 644.

Cow No. 1, born October 4, 1923, was predominantly Holstein, probably less than one-quarter Guernsey. She calved March 10, 1926, and was in her first lactation during this investigation. She was bred May 27, 1926.

Cow No. 11, born May 12, 1921, was a high-grade Holstein. She calved December 11, 1925, and was in her third lactation. She was due to calve February 27, 1927. Cows Nos. 1 and 11 were condemned for slaughter August 11, 1926, following a positive reaction to the tuberculin test. The latest previous test, January 26, 1926, had given negative results.

Cow No. 742, born December 10, 1922, was a second generation Guernsey-Holstein cross. She calved January 13, 1927, for her second lactation. She was condemned February 15, 1927, following a positive reaction to the tuberculin test. The latest previous test, December 15, 1926, was negative.

The cows were in good flesh at the beginning of the investigation and were apparently in good physical condition. Post-mortem examinations by competent veterinary clinicians showed very slight glandular lesions and that tuberculosis was present only in its early stage.

EXPERIMENTAL PROCEDURE

The cows were given their customary feed and care during a 10-day preliminary period and were milked regularly twice each day at about 6 a. m. and 6 p. m. During this period three composite samples of milk were taken for each cow. The first sample represented all the milk produced during the first four days. The second and third samples each represented all the milk produced in successive three-day periods. Following the preliminary period the cows were given water but no feed. The inanition period continued five days for cow No. 11 and six days for cows Nos. 1 and 742. The treatment of the animals, except for the absence of feed, was precisely the same as during the preliminary period.

TABLE 1.—*Composition of milk from cow No. 1 preceding and during the inanition period*

Date (1926)	Milk yield	Total solids	Fat	Protein	Lactose (gravity)	Ash	Specific gravity	Total solids	Fat	Protein	Lactose	Ash
	Lbs.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*		*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Oct. 25 [a]	[b] 13.4	12.63	4.03	3.27	4.40	0.706	1.0342	1.6924	0.5400	0.4382	0.5896	0.09460
Oct. 28 [c]	[d] 13.3	12.94	4.22	3.24	Lost.	.708	1.0356	1.7210	.5613	.4309	Lost.	.09416
Oct. 31 [c]	[d] 13.5	12.94	4.14	3.32	4.47	.716	1.0297	1.7469	.5589	.4482	.6035	.09666
Oct. 31, p. m.	13.1	12.72	4.02	3.30	4.35	.712	1.0305	1.6663	.5266	.4323	.5699	.09327
Nov. 1, a. m.	10.6	12.64	4.02	3.24	4.30	.738	1.0293	1.3398	.4261	.3434	.4558	.07823
Nov. 1, p. m.	4.4	16.28	7.53	3.85	3.80	.803	1.0277	.7163	.3312	.1694	.1672	.03533
Nov. 2, a. m.	3.3	18.04	8.70	3.50	3.10	.930	1.0283	.5953	.2871	.1155	.1023	.03069
Nov. 2, p. m.	2.0	23.12	13.34	4.97	2.60	1.063	1.0290	.4624	.2668	.0994	.0520	.02126
Nov. 3, a. m.	1.5	23.79	13.94	5.62	2.04	1.197	1.0286	.3569	.2091	.0843	.0306	.01796
Nov. 3, p. m.	.7	25.41	15.17	5.72	1.60	1.207	1.0271	.1779	.1062	.0400	.0112	.00845
Nov. 4, a. m.	.9	26.14	14.36	6.80	1.50	1.115	1.0286	.2353	.1292	.0612	.0135	.01004
Nov. 4, p. m.	.7	24.18	13.77	6.23	1.77	1.112	1.0271	.1693	.0964	.0436	.0124	.00778
Nov. 5, a. m.	.7	26.22	14.91	6.41	1.83	1.114	1.0250	.1835	.1044	.0449	.0128	.00780
Nov. 5, p. m.	.5	28.83	18.23	6.96	1.55	1.097	1.0275	.1442	.0912	.0348	.0078	.00548
Nov. 6, a. m.	.9	30.17	19.15	7.09	1.70	1.078	.0265	.2715	.1724	.0638	.0153	.00970

[a] A composite sample made by taking proportional amounts from the milk produced at each milking for 4 days.
[b] Average milk yield per milking for 4 days.
[c] Composite samples made by taking proportional amounts from the milk produced at each milking for 3 days.
[d] Average milk yield per milking for 3 days.

All samples were analyzed for percentages of total solids, fat, total protein, lactose, and ash. The methods of analysis employed by the Association of Official Agricultural Chemists were used, except that about 5 gm. of sample were employed for the fat determination by the Roese-Gottlieb method, and about 10 gm. of sample and 3 c. c. of strong nitric acid were used in the ash determination. The lactose was determined by the reduction method, the cuprous oxide being weighed directly. The specific gravities were taken at 20° C. with a chainomatic specific gravity balance.

The results obtained, including milk yields, percentages of total solids, fat, protein, lactose, and ash, and the yields of the components of the milk, are given in Tables 1, 2, and 3, and are graphically represented in Figures 1, 2, 3, and 4.

TABLE 2.—*Composition of milk from cow No. 11 preceding and during the inanition period*

Date (1926)	Milk yield	Total solids	Fat	Protein	Lactose (gravity)	Ash	Specific gravity	Total solids	Fat	Protein	Lactose	Ash
	Lbs.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*		*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Oct. 25 [a]	[b] 12.9	12.83	3.93	3.48	4.45	0.748	1.0351	1.6551	0.5070	0.4489	0.5741	0.09649
Oct. 28 [c]	[d] 13.3	12.86	3.46	3.41	Lost.	.731	1.0348	1.7104	.4602	.4535	Lost.	.09722
Oct. 31 [c]	[d] 13.9	12.90	3.84	3.49	4.56	.740	1.0301	1.7931	.5338	.4851	.6338	.10286
Oct. 31, p. m.	13.3	12.70	3.75	3.53	4.50	.729	1.0331	1.6891	.4988	.4695	.5985	.09696
Nov. 1, a. m.	11.8	13.04	4.00	3.47	4.45	.698	1.0298	1.5387	.4720	.4095	.5251	.08236
Nov. 1, p. m.	6.7	14.49	5.28	3.50	4.15	.756	1.0311	.9708	.3538	.2345	.2781	.05065
Nov. 2, a. m.	4.8	16.92	7.22	4.30	3.68	.893	1.0290	.8122	.3466	.2064	.1766	.04286
Nov. 2, p. m.	3.1	21.08	10.09	5.77	2.82	1.129	1.0341	.6535	.3128	.1789	.0874	.03500
Nov. 3, a. m.	2.6	21.98	10.67	6.14	2.77	1.220	1.0351	.5715	.2774	.1596	.0720	.03172
Nov. 3, p. m.	2.5	21.38	10.07	6.37	2.62	1.177	1.0335	.5345	.2518	.1593	.0655	.02942
Nov. 4, a. m.	2.2	23.49	11.80	6.59	2.62	1.086	1.0306	.5168	.2596	.1450	.0576	.02389
Nov. 4, p. m.	2.3	23.90	12.38	6.80	2.70	1.274	1.0342	.5497	.2847	.1564	.0621	.02930
Nov. 5, a. m.	1.6	26.48	14.88	6.88	2.55	1.225	1.0294	.4237	.2381	.1101	.0408	.01960

[a] A composite sample made by taking proportional amounts from the milk produced at each milking for 4 days.
[b] Average milk yield per milking for 4 days.
[c] Composite samples made by taking proportional amounts from the milk produced at each milking for 3 days.
[d] Average milk yield per milking for 2 days.

TABLE 3.—*Composition of milk from cow No. 742 preceding and during inanition period*

Date (1927)	Milk yield	Total solids	Fat	Protein	Lactose (gravity)	Ash	Specific gravity	Total solids	Fat	Protein	Lactose	Ash
	Lbs.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*		*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Feb. 21 [a]	[b] 20.5	14.19	5.27	3.06	4.64	0.717	1.0307	2.9090	1.0804	0.6273	0.9512	0.14698
Mar. 1 [c]	[d] 21.8	13.16	4.28	3.00	4.87	.688	1.0302	2.8689	.9330	.6540	1.0617	.14998
Mar. 4 [c]	[d] 22.6	12.96	4.18	2.93	4.80	.673	1.0298	2.9290	.9447	.6622	1.0848	.15210
Mar. 4 p. m.	22.1	12.74	4.15	2.84	4.76	.642	1.0300	2.8155	.9172	.6276	1.0520	.14148
Mar. 5 a. m.	22.9	13.22	4.69	2.73	4.55	.649	1.0259	3.0274	1.0740	.6252	1.0420	.14862
Mar. 5 p. m.	12.6	17.54	9.23	2.99	4.60	.726	1.0244	2.2100	1.1630	.3767	.5796	.09148
Mar. 6 a. m.	9.3	20.00	11.57	3.40	3.72	.804	1.0251	1.8600	1.0760	.3162	.3460	.07477
Mar. 6 p. m.	8.0	18.22	9.56	3.18	3.80	.790	1.0247	1.4576	.7648	.2544	.3040	.06320
Mar. 7 a. m.	7.6	18.22	9.72	3.30	3.78	.782	1.0219	1.3847	.7387	.2508	.2873	.05943
Mar. 7 p. m.	7.1	17.32	8.74	3.35	3.72	.828	1.0274	1.2297	.6205	.2378	.2641	.05879
Mar. 8 a. m.	4.8	19.17	10.28	3.53	3.66	.906	1.0245	.9202	.4934	.1694	.1757	.04349
Mar. 8 p. m.	7.2	16.66	8.32	2.98	3.83	.762	1.0271	1.1995	.5990	.2146	.2758	.05486
Mar. 9 a. m.	9.1	17.74	9.69	2.75	3.93	.676	1.0243	1.16143	.8818	.2502	.3576	.06152
Mar. 9 p. m.	7.4	18.44	10.11	3.04	3.79	.781	1.0226	1.3646	.7481	.2250	.2805	.05779
Mar. 10 a. m.	.5	19.06	10.13	3.34	4.07	.829	1.0214	.0953	.0509	.0167	.0204	.00414

[a] A composite sample made by taking proportional amounts from the milk produced at each milking for 4 days.
[b] Average milk yield per milking for 4 days.
[c] Composite samples made by taking proportional amounts from the milk produced at each milking for 3 days.
[d] Average milk yield per milking for 3 days.

RESULTS

The milk yield and the content of the various components showed only the normal fluctuations during the preliminary feeding period. However, after the beginning of the inanition period, the yield of milk dropped very rapidly and marked changes in the composition were found. Cow No. 1 produced an average of 13.4 pounds of milk per milking during the preliminary period. After three days without feed her production had dropped to less than 1 pound per milking and varied from 0.5 to 0.9 of a pound each milking during

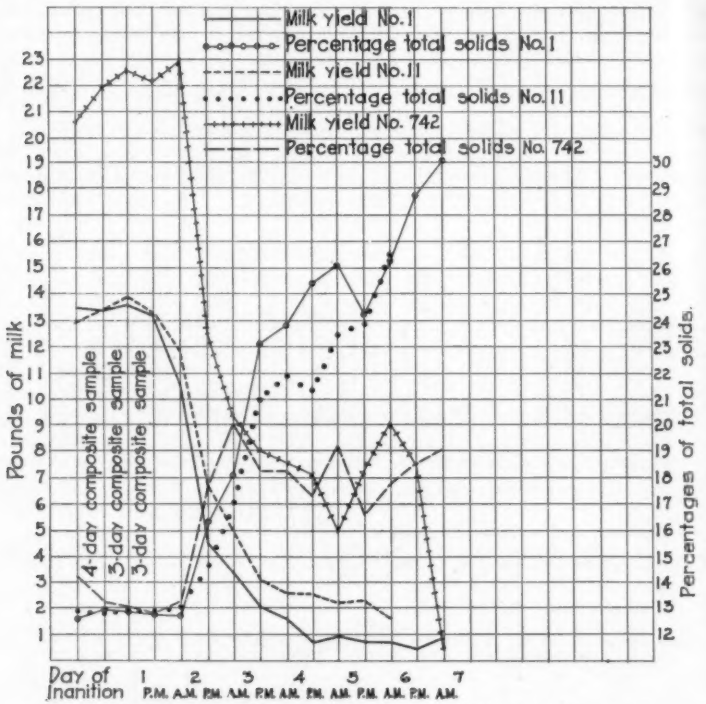

Milk yield No. 1
Percentage total solids No. 1
Milk yield No. 11
Percentage total solids No. 11
Milk yield No. 742
Percentage total solids No. 742
Pounds of milk
Percentages of total solids.
4-day composite sample
3-day composite sample
3-day composite sample
Day of Inanition
1 2 3 4 5 6 7
P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M.

FIG. 1.—Milk yields and percentages of solids preceding and during inanition period

the remainder of the period. The percentage composition of her milk also changed very radically. The total solids increased from an average of 12.82 per cent during the preliminary period to 30.17 per cent for the last milking during inanition. The fat increased from an average of 4.12 to 19.15 per cent; the protein from an average of 3.28 to 7.09 per cent; the ash from an average of 0.710 to 1.207 per cent in the seventh milking during inanition. In the remaining five milkings the ash varied between 1.078 and 1.115 per cent. The lactose averaged 4.43 per cent for seven days of the preliminary period (this determination on the second composite sample was lost) and decreased to 1.6 per cent in the seventh milking of the inanition period. It varied from 1.50 to 1.83 per cent in the remaining five milkings.

The trend of the production and of the composition of the milk was the same for cow No. 11 as for cow No. 1. The milk yield decreased from an average of 13.3 pounds per milking during the preliminary period to 2.6 pounds at the end of three days' inanition, and decreased slowly through the remaining four milkings. The percentage of total solids increased from an average of 12.86 to 26.48 per cent; fat from 3.76 to 14.88 per cent; protein from 3.46 to 6.88 per cent; and ash from 0.740 to 1.274 per cent. The lactose

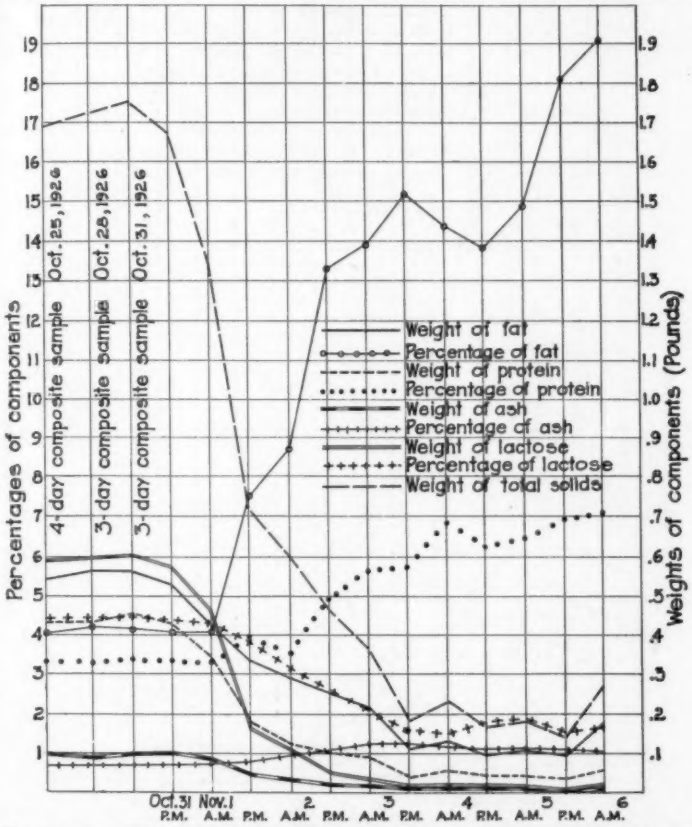
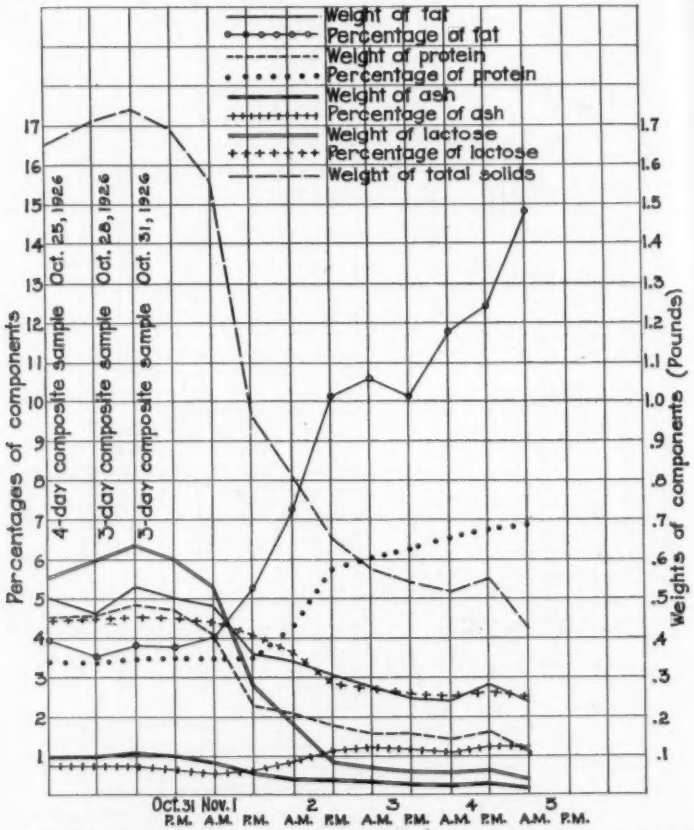

Percentages of components
Weights of components (Pounds)
4-day composite sample Oct. 25, 1926
3-day composite sample Oct. 28, 1926
3-day composite sample Oct. 31, 1926
Weight of fat
Percentage of fat
Weight of protein
Percentage of protein
Weight of ash
Percentage of ash
Weight of lactose
Percentage of lactose
Weight of total solids
Oct. 31 Nov. 1 2 3 4 5 6
P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M.

FIG. 2.—Percentages and yields of milk components for cow No. 1, preceding and during inanition period

averaged 4.50 per cent during 7 days of the preliminary 10-day period, and decreased to 2.55 per cent during inanition.

The analyses of the milk samples from cow No. 742 showed much less variation than for the other two cows. The milk yield decreased from an average of 21.5 pounds per milking during the preliminary period to 8 pounds at the end of three days' inanition, and fluctuated between 4.8 and 9.1 pounds during the next six milkings. During the last 12 hours of inanition only 0.5 pound of milk was produced, while during the preceding 12 hours the production was 7.4 pounds.

The percentage of total solids increased from an average of 13.49 to a maximum of 19.06; and the fat increased from an average of 4.63 to a maximum of 11.57 per cent at the beginning of the third day of inanition and fluctuated between 8.32 and 10.28 per cent during the remainder of the inanition period. The protein averaged 3.01 per cent during the preliminary period and varied between 2.73 and 3.53 per cent during inanition, without showing any regular increase. The lactose decreased from an average of 4.76 per cent in the pre-


Weight of fat
Percentage of fat
Weight of protein
Percentage of protein
Weight of ash
Percentage of ash
Weight of lactose
Percentage of lactose
Weight of total solids
4-day composite sample Oct. 25, 1926
3-day composite sample Oct. 28, 1926
3-day composite sample Oct. 31, 1926
Percentages of components
Weights of components (Pounds)
Oct. 31 P.M. A.M. Nov. 1 P.M. A.M. 2 P.M. A.M. 3 P.M. A.M. 4 P.M. A.M. 5 P.M.

IG. 3.—Percentages and yields of milk components for cow No. 11, preceding and during inanition period

liminary period to 3.66 per cent for the eighth milking during inanition, then increased slightly, varying from 3.79 to 4.07 per cent during the last four milkings. The value for the last milking (4.07 per cent) is, however, decidedly below the preliminary average for this cow. The ash content averaged 0.696 per cent during the preliminary period and for the most part was above this value during inanition, reaching for one milking a maximum of 0.906 per cent.

The specific gravity of the milk tended to be lower during inanition than during the normal feeding period.

DISCUSSION

The results obtained in this investigation show that inanition exerts a profound influence upon the yield and the composition of the milk of the dairy cow. As the milk production decreased the percentage content of all the components except lactose increased. The increases in percentages of fat were greatest for cow No. 1 and showed a maximum of 4.65 times the average during the preliminary 10-day feeding period. For cows Nos. 11 and 742, the increases were, respectively, 3.95 and 2.50 times the preliminary 10-day aver-

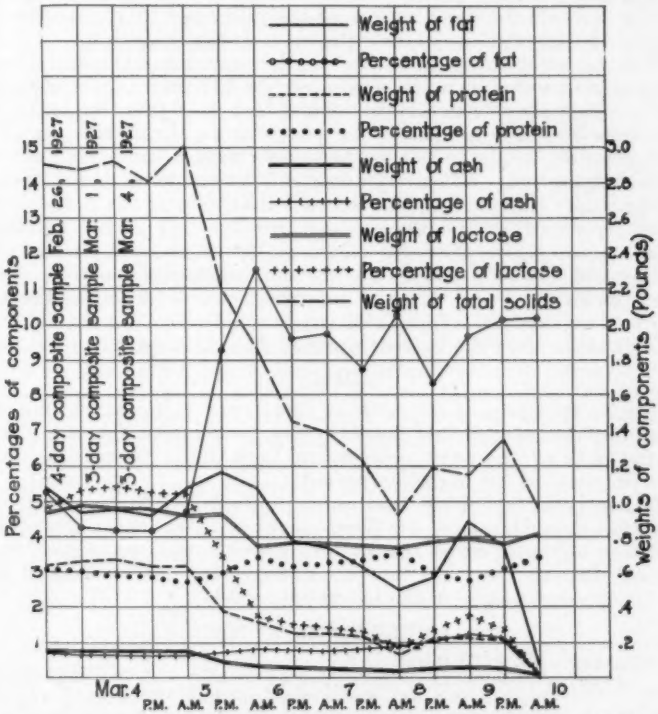

Weight of fat
Percentage of fat
Weight of protein
Percentage of protein
Weight of ash
Percentage of ash
Weight of lactose
Percentage of lactose
Weight of total solids
4-day composite sample Feb. 26, 1927
5-day composite sample Mar. 1, 1927
5-day composite sample Mar. 4, 1927
Percentages of components
Weights of components (Pounds)
Mar. 4 5 6 7 8 9 10
P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M. P.M. A.M.

FIG. 4.—Percentages and yields of milk components for cow No. 742, preceding and during inanition period

age. The maximum percentage of total solids for cow No. 1 was 2.35 times the preliminary average. For cows Nos. 11 and 742, it was, respectively, 2.06 and 1.49 times the preliminary average. The maximum percentages of protein for the three cows were, respectively, 2.14, 1.99, and 1.17 times the preliminary average. The maximum percentages of ash were, respectively, 1.70, 1.72, and 1.30 times the preliminary average.

The lactose, however, decreased from an average of 4.43 per cent to a minimum which was 33.9 per cent of that value for cow No. 1. The corresponding values for cows Nos. 11 and 742, were, respectively, 56.7 and 76.9 per cent of the preliminary average.

These changes in composition of milk, which were caused by lack of feed, agree with the observations of other investigators cited in the introduction to this paper. The reduction in the percentage of lactose also supports the statement made from this laboratory that the lactose content of milk is especially sensitive to physiological disturbances (*3*). As the quantity of lactose produced by the cows studied in this investigation decreased much more rapidly than did the volume of milk, it appears that the suggestion of Taylor and Husband (*6*) does not apply to cows subjected to inanition, since they state that the quantity of lactose elaborated by the mammary glands controls the daily volume of the milk, and that, therefore, the rate of its elaboration controls the rate of milk secretion.

The reduction in the milk flow was less rapid and the variations in composition were less marked for cow No. 742 than for the other two cows. This may be explained by the fact that this cow had begun her lactation only a short time previous to the beginning of the investigation, while the other two cows were approaching the end of their lactations. This explanation is in accord with the belief of Eckles and Palmer (*1, p. 69*), that the milk flow of the cow is stimulated by two factors chemical and nervous. It is the opinion of these investigators that the chemical factor is more or less independent of the food supply and predominates for a period of time after parturition; and that the nervous factor is almost entirely dependent on the food supply and predominates or replaces the chemical stimulus after the lactation period has attained a certain stage.

SUMMARY

The composition of the milk of three cows subjected to inanition has been determined and has been compared with the composition of the milk of the same cows when kept under normal conditions just previous to the inanition period.

It has been shown (1) that as the period of inanition progresses the milk flow decreases, the percentages of total solids, fat, protein, and ash increase, while the specific gravity and the percentage of lactose decrease, and (2) that the changes are not so marked when inanition occurs early in the lactation period.

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PARADICHLOROBENZENE AS AN ANTHELMINTIC[1]

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INTRODUCTION

Paradichlorobenzene has in recent years become well known as an insecticide. It has been found to be very effective as a moth exterminator (*3*),[2] and has been advocated as a valuable aid in controlling the peach tree borer. Some information is also available concerning its efficacy against external parasites of animals (*1*).

The chemical and physical properties of paradichlorobenzene are given by Duckett (*3, p. 6*), by Konantz (*8*), and by Sollmann (*10*). Concerning its physiological properties, Duckett (*3, p. 7*), quoting from Francis and Fortescue-Brickdale (*4, p. 99*), says that "the benzene halogen derivatives have a slight odor, are insoluble in water, volatilize without decomposition, and are very stable. * * * Corresponding to their stability it is found that the halogen is not split off in the organism, and that they do not show hypnotic properties. * * * Chlorobenzene acts on the spinal cord to a greater extent than benzene."

According to Sollmann (*10*) nothing is known of the pharmacology of paradichlorobenzene. Duckett (*3, p. 1*) quotes Curschman as saying that poisoning of human beings by paradichlorobenzene through contact with the skin is impossible and that inhalation of the vapors of this product is perfectly harmless; and, furthermore, that paradichlorobenzene is harmful to human beings only in cases of internal application of large quantities, say from 30 to 40 grains.

Sollmann (*10*) reports that dogs tolerated doses of paradichlorobenzene up to 15 gm. without showing any ill effects, but he does not consider the drug very effective as an anthelmintic.

Experiments at the Porto Rico Agricultural Experiment Station were undertaken to determine the anthelmintic value of paradichlorobenzene, especially against the hook-worm.

EXPERIMENTAL DATA

Paradichlorobenzene was administered to dogs (1) as crystals in gelatin capsules in doses of 0.1, 0.3, 0.5, and 1 gm. per kilo of body weight; (2) dissolved in liquid petrolatum or in olive oil, in doses of 1 gm. per kilo of body weight; (3) as crystals in gelatin capsules in doses of 0.5 and 1 gm. per kilo of body weight, followed an hour later by an ounce of liquid petrolatum or of castor oil; and (4) as crystals in gelatin capsules followed immediately by (*a*) a meal of fat, (*b*) a meal of whole milk, (*c*) a meal of lean meat, and (*d*) a meal of dry bread.

[1] Received for publication Apr. 6, 1927; issued November, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 649.

Liquid petrolatum and olive oil were selected as carriers because, the one being a hydrocarbon and the other a bland oil, any anthelmintic action produced would be considered as due to the paradichlorobenzene dissolved in them, since they of themselves are without any such action (*6*).

The animals were fasted overnight previous to administering the drug, and given no food for a period of two hours following dosing. The feces were then collected for a period of from 48 to 72 hours and examined. Table 1 shows the efficacy of paradichlorobenzene in experiments 1, 2, and 3.

From the table it will be seen that in the case of dog No. 26, in which the worms (*Ancylostoma caninum*) found in the large intestine were considered impotent, paradichlorobenzene, administered in the form of crystals at the rate of 0.1 gm. per kilo of body weight, was inefficacious for roundworms, tapeworms, and whipworms, and 28.5 per cent efficacious against hookworms.

Administered at the rate of 0.3 gm. per kilo of body weight, the drug was inefficacious for roundworms and whipworms, and averaged 2 per cent efficacious against hookworms, and 1.5 per cent against tapeworms, in the case of dogs Nos. 19 and 20.

Administered at the rate of 0.5 gm. per kilo of body weight, the drug was inefficacious for tapeworms, and averaged 25.45 per cent efficacious against hookworms, in the case of dogs Nos. 1, 2, and 19.

Administered at the rate of 1 gm. per kilo of body weight, the drug averaged 66.5 per cent efficacious against roundworms in two trials, and 49.5 per cent against hookworms in the case of dogs Nos. 4, 5, 9, 10, 11, 12, 15, and 16.

Administered dissolved in liquid petrolatum at the rate of 1 gm. per kilo of body weight, the drug was inefficacious for tapeworms and 4.5 per cent efficacious against hookworms, as shown in the case of dog No. 13.

Administered in olive oil at the rate of 1 gm. per kilo of body weight, the drug was 17 per cent efficacious against hookworms in the case of dog No. 14.

Administered in crystal form in gelatin capsules at the rate of 0.5 gm. per kilo of body weight, and followed an hour later by an ounce of liquid petrolatum, the drug was inefficacious for roundworms and tapeworms, and averaged 60.5 per cent efficacious against hookworms and 12.5 per cent efficacious against whipworms, in the case of dogs Nos. 22 and 24.

Administered in crystal form in gelatin capsules at the rate of 1 gm. per kilo of body weight, and followed an hour later by an ounce of liquid petrolatum, the drug was 4.5 per cent efficacious against hookworms and 100 per cent efficacious against whipworms in the case of dog No. 29.

Administered in the form of crystals in gelatin capsules at the rate of 0.5 gm. per kilo of body weight, followed an hour later by an ounce of castor oil, the drug was inefficacious for roundworms, tapeworms, and whipworms, and averaged 15 per cent efficacious against hookworms, as shown in the case of dogs Nos. 23, 25, and 27.

Administered in the form of the crystals in gelatin capsules at the rate of 1 gm. per kilo of body weight, followed an hour later by an ounce of castor oil, the drug was 62.5 per cent efficacious against hookworms in the case of dog No. 28.

TABLE 1.—*Efficacy of paradichlorobenzene, administered in several ways, as an anthelmintic for dogs*

Dog No.	Weight of dogs	Dose per kilo of body weight	Total dose	Quantity of oil	Ascarids					Hookworms					Tapeworms					Whipworms				
					Number recovered ante mortem	Number recovered post mortem			Percentage of efficacy	Number recovered ante mortem	Number recovered post mortem			Percentage of efficacy	Number recovered ante mortem	Number recovered post mortem			Percentage of efficacy	Number recovered ante mortem	Number recovered post mortem			Percentage of efficacy
						Stomach	Small intestine	Large intestine			Stomach	Small intestine	Large intestine			Stomach	Small intestine	Large intestine			Stomach	Small intestine	Large intestine	
	Kgm.	*Gm.*	*Gm.*	*Gm*																				
26	2.5	[a] 0.1	0.25	0			3		0	6		15		28.50			56		0					
19	3.0	[a] .3	.9	0			11		0			338	13	4.00			67	2	3					(b)
20	4.0	[a] .3	1.2	0								43		0			1		0					(b)
2	5.0	[a] .5	2.5	0					(b)	950		1,598	218	41.00										(b)
1	4.0	[a] .5	2	0					(b)	60	1	130	10	30.35			11		0					(b)
17	6.0	[a] .5	3	0					(b)	1	4	37	1	5.00			25		0					(b)
4	5.0	[a] 1.0	5	0						41		0		100.00			2		0					
5	6.0	[a] 1.0	6	0						28		3		90.00										
9	4.0	[a] 1.0	4	0				2	100			9	26	75.00										
10	3.5	[a] 1.0	3.5	0						102		10		91.00										
11	7.0	[a] 1.0	7	0						63		189	12	24.25			1							
12	2.0	[a] 1.0	2	0	1		4	1	33	6		231		2.60					0					
15	4.5	[a] 1.0	4.5	0								19		0			6		0					
16	6.0	[a] 1.0	6	0						4				12.50					0					
13	4.0	[c] 1.0	4	15					(b)	5	4	357	12	4.50			10		0					(b)
14	4.0	[d] 1.0	4	15					(b)	6		183	31	17.00					(b)					(b)
22	3.0	[e] .5	1.5	30			3		0	37		0		100.00			1		0					
24	3.5	[e] .5	1.75	30						54		392	51	21.00					(b)	1			3	25
29	4.0	[e] 1.0	4	30					(b)	5	2	106		4.50						1				100
23	3.5	[f] .5	1.75	30			3		0			11		0										
25	4.0	[f] .5	2	30		12				66		817	298	30.00			1		0				6	0
27	8.0	[f] .5	4	30						7	1	42	1	15.00										
28	5.0	[f] 1.0	5	30					(b)	5		3		62.50					(b)					

[a] Administered as crystals in gelatin capsules.
[b] None found.
[c] Dissolved in liquid petrolatum.
[d] Dissolved in olive oil.
[e] Gelatin capsules followed in an hour by liquid petrolatum.
[f] Gelatin capsules followed in an hour by castor oil.

TOXICITY

Dogs Nos. 9 and 10 died showing pronounced symptoms of intoxication. The practice of the experiment station has been to fast the animals overnight before administering paradichlorobenzene, and to withold feed for a period of two hours following dosing. In the case of dogs Nos. 9 and 10, however, the attendant inadvertently gave the animals fat meat immediately after dosing.

The dogs received the drug at about 9.30 a. m., and were down and trembling violently at 1 p. m. Dog No. 9 soon became unconscious and died about 36 hours later without having regained consciousness. Dog No. 10 was not so violently affected, but showed incoordination of movement after 24 hours, walking with an irregular gait or standing with its legs spread far apart.

To learn whether this result was due to the absorption of paradichlorobenzene, dogs Nos. 18 and 21 were given the drug at the rate of 0.5 gm. per kilo of body weight. Dog No. 18 received a piece of fat immediately following dosing, and dog No. 21 was given all the whole milk it would take.

Both dogs showed decided symptoms of intoxication at 1 p. m., but the symptoms were not nearly so pronounced as in the case of dogs Nos. 9 and 10. The symptoms were still evident at 4 p. m. but disappeared before the next morning.

Four days later the experiments were repeated with the same dogs. The same amount of paradichlorobenzene was given as before, but in addition dog No. 18 received lean meat and dog No. 21 dry bread and water. The drug was administered at 9 a. m., and the first symptoms of intoxication were visible at 1 p. m. These passed off rapidly, however, leaving the animals with no apparent permanent ill effect.

Dog No. 21 passed eight ascarids after receiving the first dose of paradichlorobenzene on October 13, and died a few days after receiving the second dose on October 17. No ascarids were found post-mortem, proving the drug to be 100 per cent efficacious against these intestinal worms in this instance. No percentage of efficacy against hookworms was determined in the case of dog No. 21.

EFFECT ON EDIBILITY OF MEAT ANIMALS

In connection with the experiments with paradichlorobenzene as an anthelmintic the drug was observed to render unfit for human consumption the meat of animals to which it was administered.

Previous to the arrival of the author at the Porto Rico Experiment Station paradichlorobenzene in crystal form had been administered to pigs. One pig, which had received a total of 14 gm. of the drug during a period of four months, was sold on the hoof, that is, before slaughter, as is the local custom. Those who purchased the meat could not eat it and were refunded their money. The meat smelled and tasted of paradichlorobenzene. The second pig, which had been kept as a check on the first experiment, was given 5 gm. of the drug in one dose and was not slaughtered until two weeks later. The meat of this animal also proved to be unfit for human consumption.

One authority reports tasting paradichlorobenzene in the eggs of hens which were fed corn that had been fumigated with the drug, To test the accuracy of the report the author, about December 27, 1925, fed to some of the hens at the station the ground liver of a dog

to which paradichlorobenzene had been administered. Up to and including January 9, 1926, paradichlorobenzene could be tasted in the yolks of the eggs laid by these hens.

CONCLUSIONS

Administered in doses of 0.1, 0.3, 0.5, and 1 gm. per kilo of body weight in bland oils and in crystal form, followed by a bland oil in some instances, and by a purgative oil in others, paradichlorobenzene is somewhat efficacious against intestinal worms.

The results obtained were not sufficiently uniform to permit classifying paradichlorobenzene as an effective anthelmintic, and are in accordance with the findings of Sollmann (*10*).

The results from using paradichlorobenzene dissolved in olive oil and alone are in agreement with the opinion expressed in 1918 by Hall (*5*) that "olive oil seemed to be decidedly contraindicated in [connection] with anthelmintics."

Hall and Foster (*6, p. 432*) also point out the fact that liquid petrolatum seriously diminished the efficacy of chenopodium, which seems to be true also in the case of paradichlorobenzene.

The results apparently confirm also the hypotheses advanced by Caius and Mhaskar (*2*) and Hall and Shillinger (*7*) concerning the relationship of the chlorine content and the solubility of halogen compounds to their anthelmintic efficacies. Paradichlorobenzene contains only 2 atoms of Cl, is practically insoluble in water, and theoretically therefore should be inferior to such compounds as carbon tetrachloride and the new anthelmintic tetrachlorethylene, which contain a larger number of chlorine atoms and are more soluble in water.

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CYTOLOGICAL STUDIES ON VIRUS DISEASES OF SOLANACEOUS PLANTS [1]

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INTRODUCTION

Microscopical examinations of the tissues of mosaic-diseased plants have been made by numerous investigators since the earliest recognition of diseases of the plant virus group. With a few exceptions, these investigations have been confined to the study of a single virus disease upon a single species of host. It has now, however, come to be recognized that there are a number of different virus diseases due to distinct and specific viruses, which frequently have an extended host range, and several of which may be capable of infecting the same host plant. In certain instances, where a single plant species or group of species is susceptible to a number of such diseases, considerable difficulty may be experienced in distinguishing clearly between the latter, though in some cases this may be accomplished by means of a comparative study of the host range, symptomatology, and certain other properties of the viruses concerned. It was believed that a comparative cytological study of such a group of diseases upon an extended host range would be of value in determining whether any microscopical features were present which could be correlated with any specific viruses, thereby providing an additional means of identification. Such a study might also be expected to throw further light on the possible relationships of the different viruses. It was especially borne in mind that various investigators have reported the occurrence of vacuolate, protoplasmic bodies in the tissues of certain mosaic-diseased plants, and that there are those who believe these bodies to be a form of protozoan or other living organism and the casual agent of the disease in question. It is therefore of considerable importance to ascertain to what extent such bodies are associated with virus diseases in general, and a cytological study of the nature described should be of value in this connection. A number of virus diseases were available for study on a variety of solanaceous plants, and the results of a cytological examination of these are presented in this paper, the first section dealing with diseases of a group of solanaceous plants, excluding the potato, and the second with diseases of the potato only, most of which are at present believed to be limited to the potato as host.

LITERATURE REVIEW

There is considerable literature dealing with the histological and cytological aspects of plant virus diseases. This has been reviewed in detail by various writers, notably Goldstein (*8*)[3] and Smith (*31*), and

[1] Received for publication July 14, 1927; issued November, 1927.
[2] The thanks of the writer are due Dr. James Johnson, under whose direction this work was carried out, both for his valuable cooperation in supplying the material for examination and for his interest and advice throughout the course of the investigation. The writer is also indebted to Prof. L. R. Jones for his interest in the work and for his helpful criticism.
[3] Reference is made by number (italic) to "Literature cited," p. 669.

in the present paper it is proposed to give only a brief consideration to the more important publications.

Histological modifications occurring in mosaic tissues were first described by Woods (*34*) in connection with tobacco mosaic. This investigator observed a reduction in length of the palisade cells in the lighter areas of the leaf and a decrease in the amount of intercellular space throughout these regions. Iwanowski (*10*) later confirmed these observations, describing also certain variations in the width of the lamina in the light and dark areas, together with certain changes in the chloroplasts and nuclei. In the dark green areas the palisade tissue was found to consist to two layers of cells.

Similar modifications have since been reported by different investigators in association with various other mosaic diseases, namely, mosaic of cucumber (*4*), potato (*25*), tomato, petunia, henbane, pepper, raspberry, pea, bean, clover (*3*), pecan (*28*), sugar cane (*2, 24*), *Datura stramonium*, pokeweed, and Aquilegia sp. (*31*).

The presence of cell inclusions in mosaic tissues was first reported by Iwanowski (*10*), who described bodies of three types occurring in the chlorotic areas of mosaic tobacco leaves: (1) Colorless, crystalline plates or layers, resembling some waxy material but of lower refractive index, and showing cross-striations on treatment with acids; (2) protoplasmlike bodies, near, or in close connection with, the nuclei, and whose appearance suggested parasitic amoebae (these bodies were apparently distinct from the cell cytoplasm, and were considered as some reaction product of the cells); (3) granular inclusions, which, on staining, resembled zoogloea, consisting of very minute, short rods, usually occurring in the palisade cells and believed to be bacteria.

Inclusions more or less similar to the amoebalike bodies of Iwanowski have since been described by various workers in association with several different virus diseases of plants. Lyon (*20*) reports the presence of such bodies in the galls produced in Fiji disease of sugar cane,[4] and these are described more fully by Kunkel (*17*). Both authors believe the bodies to be living organisms. Kunkel gives a detailed account of somewhat similar bodies present in mosaic corn (*15*), *Hippeastrum equestre* (*16, 18*), Chinese cabbage (*18*), and sugar cane (*19*). Palm (*27*) gives a fuller description of the vacuolate bodies associated with tobacco mosaic and describes also certain minute granules in the mosaic tissues which he believes to be the causal organism. McKinney, Eckerson, and Webb report the presence of intracellular bodies, somewhat similar to those of mosaic corn, in rosette and mosaic wheat (*23*) and in mosaic *Hippeastrum johnsonii* (*22*). K. M. Smith (*32*) finds amoebalike bodies in mosaic potato, which he considers to be "some degeneration product of the cell," and Goldstein (*9*) describes protoplasmic inclusions associated with "mosaic diseased" and "dwarfed" dahlias. Further descriptions of the different cell inclusions occurring in mosaic tobacco are given by Rawlins and Johnson (*29*) and by Goldstein (*7, 8*). F. F. Smith (*31*) reports the presence of protoplasmic bodies in the tissues of mosaic tobacco, petunia, *Datura stramonium*, and pokeweed, and in *Euonymus japonicus* affected with an infectious chlorosis.

Several other types of inclusion have been described as associated with certain mosaic tissues. Sorokin (*33*) describes the formation of

[4] This may or may not be a virus disease

blisterlike spheres from the chloroplasts of mosaic tomato leaves and the occurrence of minute granular bodies in the tissues. Eckerson (*5*) reports the presence of small bodies, believed to be flagellates, in mosaic tomato, wheat, *Hippeastrum johnsonii*, dahlia, and squash, and Klebahn (*14*) finds, in the young phloem cells of *Anemone nemorosa* affected with the disease "Alloiophyllie," minute bodies which he considers to be living organisms.

VIRUS DISEASES OF SOLANACEOUS PLANTS, EXCLUDING THE POTATO

MATERIAL

The material chosen for microscopical examination was selected from a wide range of solanaceous plants of different genera and species affected with one or more of 11 different virus diseases. These diseases have been fully described by Johnson (*11*, *13*), as regards differential host symptoms and other differential properties of the viruses concerned, under the following names: Tobacco mosaic, cucumber mosaic, speckled tobacco mosaic, mild tobacco mosaic, spot necrosis, ring spot, yellow tobacco mosaic, medium tobacco mosaic, bleaching mosaic, tomato stem necrosis, and petunia mosaic. Since the work was carried out in association with Johnson, there is no doubt about the identity of the diseases studied so far as his proposed classification is concerned, and the descriptions of them need not be repeated here. The more common host plants studied are listed in Table 1.

The method employed in these investigations was to inoculate each virus simultaneously to five plants of each host species under study, the individual plants of each species being of the same age and approximately the same size and vigor. The plants were inoculated when quite young and in a rapidly growing condition. Five similar but uninoculated plants served as a control in each trial. All trials were conducted in one greenhouse run at a temperature of 27° to 32° C. When the symptoms of disease were fully developed, material for microscopical examination was carefully chosen in each case from individual leaves of plants which showed the most intense mottling, chlorosis, or other symptoms of disease. At the same time healthy material for comparison was obtained from the control plants. It has been found by various workers, and has been observed by the writer during the present investigation, that the amount of internal modification or variation in mosaic-diseased plants is in general proportional to the intensity of external symptoms, and is more pronounced and varied in the leaves than in any other organ of the plant. Material was therefore selected which might be expected to show as fully developed internal symptoms of disease as possible for any host species in respect to each individual virus, and material which could be considered in all cases as truly comparative. Certain combinations of host and virus which produced only very faint or no symptoms of mottling were frequently not included in the examination. In some cases it was possible to secure material from only a single set of inoculations, though usually several sets were available for study at different times.

The leaf symptoms produced on the different hosts by different viruses were very varied, and Figure 1 illustrates some of the types of mosaic pattern secured which were characteristic of different viruses on tobacco and which were included in the present examination.

METHODS

The present examination was conducted largely upon material that had been fixed and stained by the usual methods, though fresh unstained material was also studied from time to time by means of

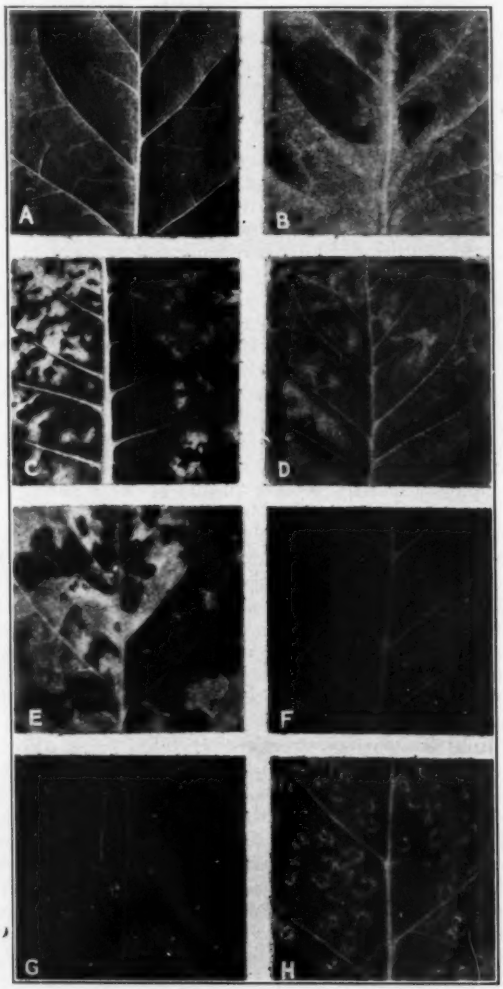


FIG. 1.—Types of mosaic pattern produced on tobacco by different virus diseases: A, Healthy tobacco; B, cucumber mosaic; C, tobacco mosaic; D, tobacco mosaic attenuated by heat; E, yellow tobacco mosaic; F, speckled tobacco mosaic; G, spot necrosis; H, ring spot

free-hand sections to serve as a check upon the other. Small portions, usually square or triangular in shape, with sides measuring a few millimeters, were cut out from the leaf lamina with a sterile safety-

razor blade, placed immediately in the fixing fluid and embedded in the usual way. If the leaves showed any form of mottling, portions were cut out from both the chlorotic and the darker areas and examined separately. Considerable difficulty was experienced in finding a fixing fluid that was satisfactory for all purposes. Many were tested, including Flemming's fluids, chrom-acetic solutions of different strengths, Juel's, Bouin's, and Zenker's fixatives, acetic alcohol, formol alcohol, formol acetic alcohol, Gilson's and other sublimate fixatives and Carnoy's fluid. Several of these gave excellent results with material from young leaves, but proved very unsatisfactory for older tissues. For general purposes formol acetic alcohol (100 parts 50 per cent alcohol, 6.5 parts formalin, and 2.5 parts glacial acetic acid), used for 24 to 48 hours, was considered most suitable and was employed extensively. Carnoy's fluid (6 parts absolute alcohol, 3 parts chloroform, and 1 part glacial acetic acid), used for about 15 minutes, was found to cause less distortion of the cells and was employed for a time, but was subsequently abandoned on account of its rather severe action on the cell contents and of the less satisfactory staining properties which it conferred upon the tissues.

The material was sectioned at a thickness of 7 microns in a plane perpendicular to the leaf surface, and stained with Haidenhain's iron-alum haematoxylin (iron-alum 2 per cent aqueous about 3 hours, haematoxylin ½ per cent aqueous about 2 hours, destained in iron-alum 1 per cent aqueous). The slides were examined with a 2 mm. apochromatic oil immersion lens and 7.5× compensating oculars. All photomicrographs were made from stained slides with the 2 mm. lens and a 6× ocular. All drawings were made with the aid of a camera lucida, using the 2 mm. lens and a 7.5× ocular.

The amount of material examined for any given virus on any one host varied considerably according to the amount that was available for study and according to certain other considerations. The minimum, however, consisted of four or five leaf portions from the chlorotic areas and about as many from the dark green areas, these being taken from several different leaves and usually from several plants. From each leaf portion, three or more microscopic slides were prepared, 50 or more sections being mounted on each slide. Usually, however, considerably more material than this was examined, and sometimes a great deal was worked through. For example, with petunia mosaic on tobacco, 14 leaf portions from 5 different plants were examined, and with speckled tobacco mosaic on the same host, 15 leaf portions from 4 plants.

RESULTS

Certain modifications were observed in the diseased tissues which were of more or less general occurrence throughout the material examined and were not limited to any specific hosts or viruses, though varying according to the intensity of external symptoms developed on the leaves. Similar modifications have already been described by Woods (*34*), Iwanowski (*10*) and other investigators, in relation to a number of other mosaic diseases, and these appear to be characteristic of such disease in general. Such modifications relate to changes in the histological structure of the leaf tissues and in the chloroplasts and nuclei.

In the earliest stages of development of the disease, or in other cases where the color modifications occurring in the leaves were only faintly developed, the chloroplasts in the lighter regions appeared somewhat paler and more yellow than those of the normal leaf, indicating some change in the composition of the chlorophyll. In the fixed material this was indicated by a difference in the staining capacity of the chloroplasts, those in the paler areas taking on a somewhat lighter stain. Where the external symptoms of mottling or chlorosis were marked, the chlorophyll had changed from green to yellow, and the plastids were reduced in size, in severe cases of disease sometimes disintegrating and apparently breaking up into small particles. In leaves infected when very young, the chloroplasts did not develop normally, but remained small and fewer in number than in the healthy cell. When placed in water, these plastids often swelled up to form colorless vesicles, as described by Iwanowski (*10*), and it is thought possible that this may have some bearing on the formation of the "spheres" reported by Sorokin (*33*). In the dark green areas, on the other hand, the chloroplasts always appeared very large, frequently more numerous, and of a somewhat deeper green than normal.

Modifications in the form of the cells occurred wherever the leaves showed a marked degree of mottling or chlorosis. According to the intensity of such symptoms, the palisade cells in the yellow areas appeared shorter in length and somewhat broader than normal, or reduced to a cuboidal form, while those of the darker areas appeared somewhat longer and thinner than normal and very closely crowded together. In the most severe cases, the palisade tissue of the darker areas consisted of two layers of cells. These modifications resulted in variations in the thickness of the leaf lamina, the lighter areas being reduced in some cases to about two-thirds the thickness of the darker areas.

Similar histological changes and modifications in the chloroplasts were associated with the different potato virus diseases, which will be considered in a later section.

In the more severe forms of disease, the host nuclei were frequently affected, those in the lighter areas of the leaf often appearing shrunken and distorted, and staining more lightly than normal; at other times appearing considerably swollen, with enlarged nucleoli. This latter type was especially characteristic of the potato mosaics. In certain material the nuclei contained square or oblong plates of some dark-staining material, whose nature was not determined, and which, when viewed on end, appeared as narrow rods or bands. Usually one or two of these plates were present in a single nucleus, which contained in addition an apparently unaltered nucleolus; such nuclei were very abundant in some material. They were associated with mild tobacco mosaic on all hosts examined with the exception of *Nicotiana glutinosa*, with bleaching mosaic on pepper and tomato, and with tobacco mosaic on henbane. Their significance is not understood.

It can not, however, be said that any of these modifications occurring in the diseased tissues are sufficiently characteristic of any particular virus or group of viruses to be of value for diagnostic purposes. Their chief significance no doubt lies in the indication which they give that the diseases in question are probably closely related one to another. On the other hand, a study of the association of cell inclusions with the different viruses reveals quite another situation.

In the case of ordinary tobacco mosaic on tobacco (*Nicotiana tabacum*), cell inclusions of two types were found to be invariably present in the chlorotic areas of mottled leaves, namely, the protoplasmic, vacuolate bodies previously described by various writers and which, for convenience, will be referred to as "x-bodies," [5] and the "striate material" of Rawlins and Johnson (*29*), which, according to Iwanowski (*10*), Goldstein (*7*), and others, results from the action of acids in the fixing fluid on certain waxy-crystalline plates occurring in the living, diseased cells. The appearance of the x-bodies in the stained sections of mosaic tobacco leaves is shown in Plate 1, A, B, C, *b*. The small, dark-staining bodies described by Rawlins and Johnson (*29*) were observed only rarely, probably because the material was usually taken during later stages of development of the disease. Contrary to the observations of Rawlins and Johnson (*29*) and of Smith (*31*), both x-bodies and striate material appeared to occur as commonly in the field as in the greenhouse. Material taken from 20 different tobacco plants growing in the field, and several feet in height, contained in every case abundant inclusions of both types.

TABLE 1.—*Occurrence of cell inclusions in various solanaceous host plants in association with different viruses*

Disease	Virus	Hosts												
		Nicotiana tabacum	Nicotiana glauca	Nicotiana glutinosa	Capsicum annuum	Lycopersicon esculentum	Physalis pubescens	Physalis franchetti	Petunia violacea	Hyoscyamus niger	Nicandra physaloides	Solanum laciniatum	Solanum mini-atum	Solanum atropurpureum
Tobacco mosaic	Tobacco virus 1	++	0	0	++	++	++	++	++	++	++	0	++	++
Cucumber mosaic	Cucumber virus 1	0	0	0	0	0	0	----	0	0	----	0	----	0
Speckled tobacco mosaic	Tobacco virus 2	0	----	----	----	0	----	----	0	0	----	----	----	----
Mild tobacco mosaic	Tobacco virus 3	0	----	0	0	0	0	----	0	0	----	----	----	----
Spot necrosis	Tobacco virus 4	0	----	----	----	0	0	----	0	0	0	----	0	0
Ring spot	Tobacco virus 5	0	----	----	----	0	0	----	----	0	----	----	0	----
Yellow tobacco mosaic	Tobacco virus 6	+	+	----	----	++	++	+	++	++	----	----	----	0
Medium tobacco mosaic	Tobacco virus 7	----	----	----	----	+	+	----	+	++	----	----	----	0
Bleaching mosaic	Tobacco virus 8	0	0	----	0	0	0	----	0	0	----	----	----	----
Tomato stem necrosis	Tobacco virus 9	0	0	----	----	0	0	----	0	0	----	----	----	0

++ indicates that x-bodies and striate material are both present. + indicates striate material only present and 0 indicates no inclusions present.

On inoculation of the tobacco-mosaic virus to other solanaceous hosts, these two types of inclusion were again produced in every case where definite mottling or chlorosis of the leaves was secured, and were invariably present in the lighter colored areas, though never occurring in the dark green parts. Table 1 shows a number of host plants examined which were affected with tobacco mosiac, and the inclusions present in each case. In addition to the hosts listed in this table, six others were examined which were affected with tobacco mosaic, and x-bodies and striate material were found to be associated with each. These hosts were: *Physalis alkekengi*, *Solanum cabiliense argenteum*, *S. marginatum*, *S. nigrum*, *S. pyracanthum*, and potato

[5] This term was introduced by Goldstein (*7*).

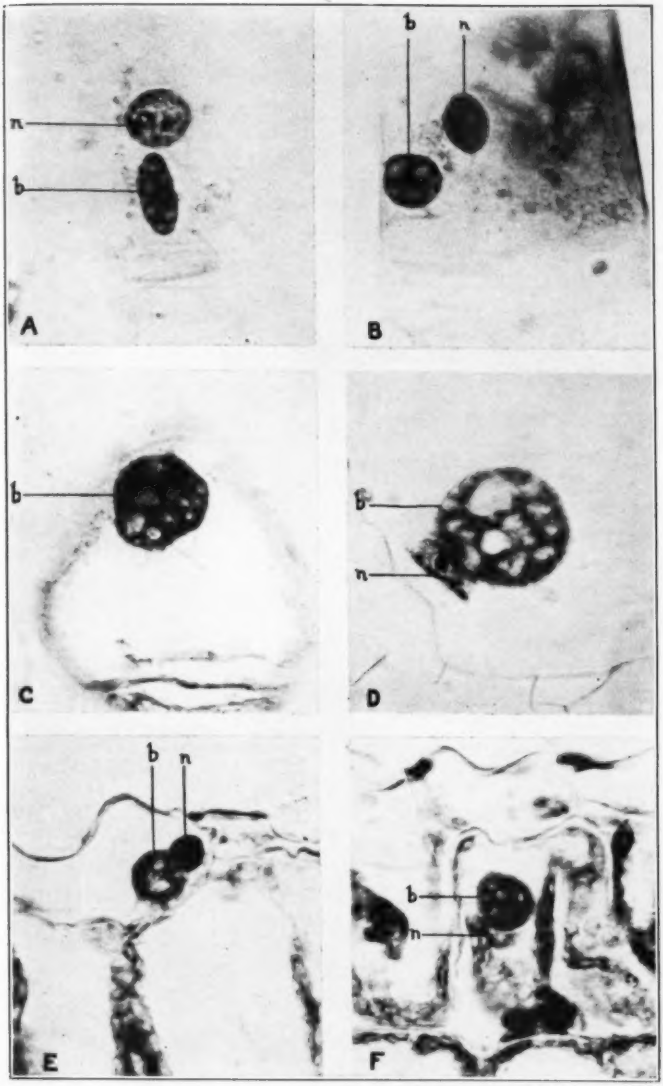
(Rural New Yorker and Green Mountain varieties). (Pl. 2, F, and fig. 2, E, J.)

TABLE 2.—*Summary of results showing the occurrence of cell inclusions in association with different viruses on all hosts examined*

Disease	Virus	Number host species examined	Number showing x-bodies and striate material	Number showing striate material only	Number showing no inclusions
Tobacco mosaic	Tobacco virus 1	19	16	0	3
Cucumber mosaic	Cucumber virus 1	11	0	0	11
Speckled tobacco mosaic	Tobacco virus 2	4	0	0	4
Mild tobacco mosaic	Tobacco virus 3	7	0	0	7
Spot necrosis	Tobacco virus 4	9	0	0	9
Ring spot	Tobacco virus 5	5	0	0	5
Yellow tobacco mosaic	Tobacco virus 6	9	4	4	1
Medium tobacco mosaic	Tobacco virus 7	5	1	3	1
Bleaching mosaic	Tobacco virus 8	7	0	0	7
Tomato stem necrosis	Tobacco virus 9	7	0	0	7
Petunia mosaic	Petunia virus 1	1	0	0	1

In all, out of 19 host species examined, 16 showed inclusions of both types. (Table 2.) Of the remaining 3 examined, which contained no inclusions, 1 (*Nicotiana glauca*) showed no symptoms of any kind except a general stunting of the plants, 1 (*N. glutinosa*) showed stunting and necrosis but no traces of mottling or chlorosis, and 1 (*S. laciniatum*) showed only a very faint mottling. Moreover, the inclusions, if present, were usually very abundant in the tissues and occurred in all parts of the leaf lamina except in the vascular bundles. In pepper (*Capsicum annuum*), however, where only a general chlorosis of the leaves was produced, without any distinct mottling, the striate material was abundant throughout the tissues, but the x-bodies were present only in the cells adjacent to the veins. In all hosts both types of inclusion were closely similar to those occurring in tobacco, resembling them in form, general appearance, staining properties and, except in pepper, in general distribution in the leaf. In some hosts, however, the x-bodies did not reach quite as large a size as in tobacco. It is interesting to note that *P. alkekengi*, once reported as a symptomless carrier (*26*), but which developed a definite mottling after inoculation with tobacco mosaic in the writer's trials, contained both x-bodies and striate material in the cells. Tobacco plants inoculated with a combination of tobacco and cucumber mosaic viruses, or with a combination of tobacco mosaic virus and six other viruses, also developed inclusions of these two types, as did tobacco plants inoculated with tobacco mosaic virus which had been attenuated by heat (*12*). Similar inclusions have been described by Goldstein (*7*) in *S. sculeatissimum* affected with tobacco mosaic. It is, therefore, evident that these two types of cell inclusion, namely, x-bodies and striate material, already known to be associated with tobacco mosaic on tobacco, must be considered as a constant feature of this disease irrespective of host plant, occurring wherever a definite mottling or chlorosis is produced.

On the other hand, no cell inclusions were observed in any healthy plant, although material from mosaic-free individuals of each host species under consideration was always examined at the same time as material from mosaic plants.



Photomicrographs of cell inclusions present in the leaves of certain mosaic-diseased plants. × 785

A.—Tobacco mosaic on tobacco. X-body (*b*) and nucleus (*n*) in a hair cell.

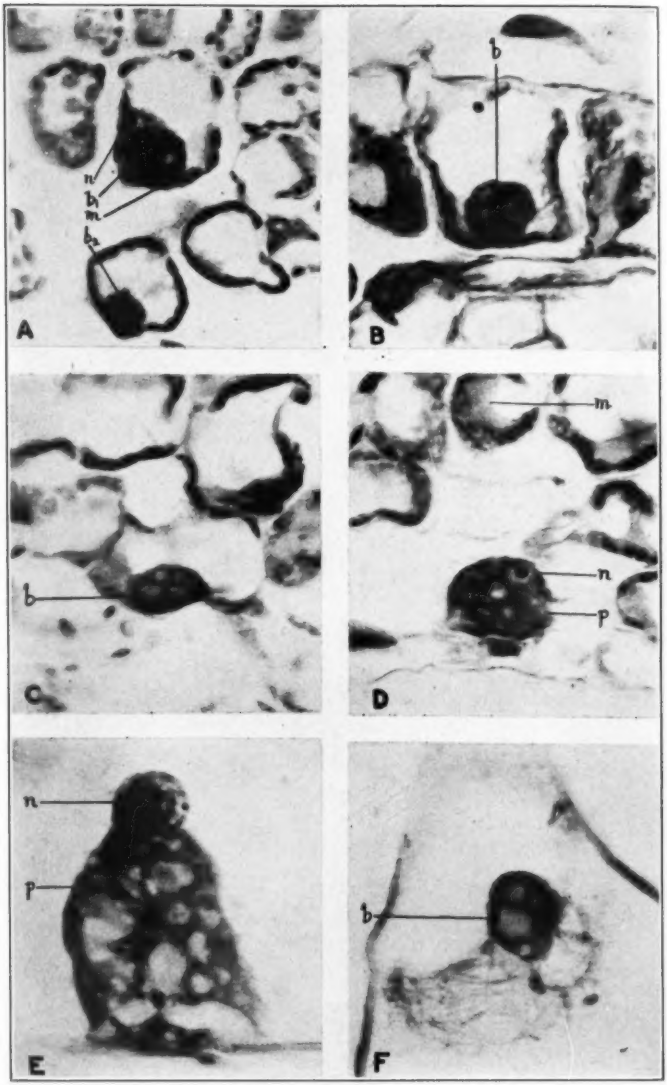
B.—Tobacco mosaic on tobacco (nucleus slighty out of focus).

C.—Tobacco mosaic on tobacco. Large x-body (*b*) in a hair cell. This was one of the largest observed in this type of material.

D.—Yellow tobacco mosaic on petunia. Very large, coarsely granular body (*b*) in contact with slightly crushed nucleus (*n*).

E.—Crinkle mosaic on Green Mountain potato. Body (*b*) and nucleus (*n*) in epidermal cell.

F.—Crinkle mosaic on Green Mountain potato. Body (*b*) and part of nucleus (*n*) in palisade cell.



Photomicrographs of cell inclusions in the leaves of potato in association with certain virus diseases. × 785

A.—Crinkle mosaic on Green Mountain Potato. Bodies (b_1 and b_2), nucleus (n) and brown material (m) in mesophyll cell.

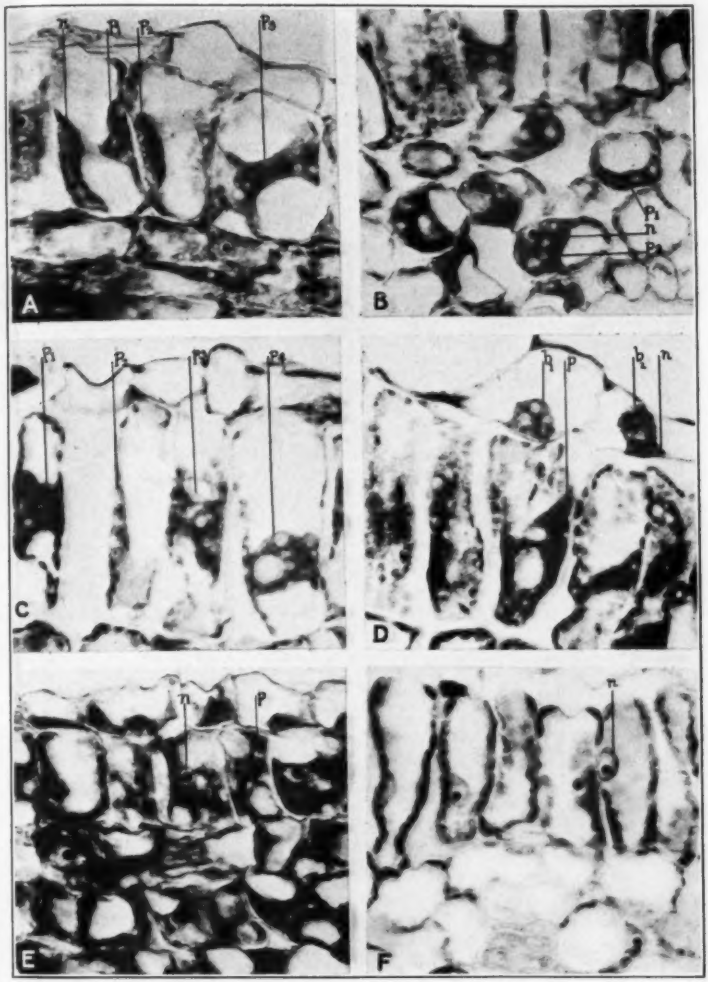
B.—Crinkle mosaic on Green Mountain potato. Body (b) in palisade cell.

C.—Rugose mosaic on Green Mountain potato. Body (b) in mesophyll cell.

D.—Rugose mosaic on Green Mountain potato. Mesophyll cell filled with vacuolate material (p); m, brown material.

E.—Supermild mosaic on American Wonder Potato. Large mass of vacuolate material (p) and nucleus (n) in hair cell.

F.—Tobacco mosaic on Rural New Yorker potato. X-body (b) in hair cell.



Photomicrographs of vacuolate, protoplasmlike material in the leaves of potato in association with certain virus diseases. × 590

A.—Crinkle mosaic on Green Mountain potato. Vacuolate material in palisade cells as an irregular layer or mass adjacent to the wall (p_1, p_2), or as a bridge across the cell (p_3); n nucleus.

B.—Crinkle mosaic on Green Mountain potato. Vacuolate material in mesophyll cells, forming a layer adjacent to the wall (p_1), or an irregular mass (p_2); n nucleus.

C.—Crinkle mosaic on Green Mountain potato. Vacuolate material in palisade cells as a bridge across the cell (p_1, p_2), a layer against the wall (p_3), or a partially rounded mass (p_4).

D.—Crinkle mosaic on Green Mountain potato. Vacuolate material as an indefinite mass (p) or as definite bodies (b_1, b_2); n nucleus.

E.—Rugose mosaic on Bliss Triumph potato. Abundant vacuolate material (p) in the cells; n nucleus.

F.—Healthy Green Mountain potato. No vacuolate material present; n nucleus.

In contrast with tobacco mosaic, no inclusions of any type were found to occur in association with cucumber mosaic when inoculated to the same hosts as tobacco mosaic. Cucumber mosaic was studied altogether on 10 solanaceous host species (Table 1), in 7 of which x-bodies and striate material were produced in abundance by tobacco mosaic; in the case of cucumber mosaic, however, cell inclusions were entirely absent from the host tissues, although on many hosts quite as marked symptoms of mottling were developed as were produced by tobacco mosaic. For example, on young tobacco plants, cucumber mosaic caused a very striking chlorosis which was quite as intense as the chlorotic areas developed by tobacco mosaic on this host (fig. 1, B, C), yet although a considerable amount of material was examined, neither x-bodies nor striate material were ever observed in association with cucumber mosaic. Young cucumber plants affected with this mosaic were also examined, but again no inclusions were observed. In order to determine whether x-bodies or other cell inclusions were associated with this disease, but were very slow in developing, material was also examined from certain leaves of tobacco, pepper, and cucumber which had already shown marked mottling for several weeks. No inclusions, however, were observed. This point is of particular interest since it was formerly believed by some investigators that cucumber and tobacco mosaic were identical, owing to the fact that they frequently produced very similar symptoms on tobacco. Cytological considerations, however, support the evidence derived from other studies that these two diseases are due to two different viruses.

As with cucumber mosaic, no inclusions were found to occur in association with seven other virus diseases of solanaceous plants, namely, speckled tobacco mosaic, mild tobacco mosaic, spot necrosis, ring spot, bleaching mosaic, tomato stem necrosis, and petunia mosaic, although, with the exception of the last named, these diseases were studied on a variety of host species (Tables 1 and 2), and the symptoms of mottling produced were always quite definite and frequently very striking[6] (fig. 1, F, G, H). Petunia mosaic was studied only on tobacco; considerable material was, however, examined, and the evidence is therefore considered as conclusive in this case also.

In connection with these seven virus diseases, it may perhaps be maintained that negative evidence is never wholly convincing, and that inclusions may have been present in some tissues although not detected. It is believed, however, that by the methods employed this possiblity has been eliminated. By the method of staining used, the x-bodies showed up conspicuously in the finished slides as blue-gray to deep blue-black objects. They presented a very different appearance from the host nucleus and could be readily distinguished from this, as is apparent from Plate 1, A, the bodies appearing of a homogeneous and uniformly stained material with vacuoles, and the nuclei as very granular, almost colorless bodies containing numerous dark chromatin granules, and immediately recognizable by the conspicuous, deep black nucleoli. The x-bodies were also readily distinguishable from the chloroplasts, which stained more lightly than

[6] In addition to the host plants shown in Table 1, spot necrosis was examined on *Solanum robustum* and no inclusions were found.

the former, besides differing in shape, size, and structure; and with a little practice it became possible to detect the x-bodies in the tissues, if present, with ease and rapidity. Similarly, the striate material was very conspicuous and could not be confused with any other constituent of the cell. Moreover, when inclusions were present at all in any material, even if occurring but rarely, several at least could be detected in almost any section examined, and with certainty in every slide. Hence, as it was customary to mount, on an average, 50 or more sections per slide and to prepare three or more slides from each portion of leaf material examined, the possibility of inclusions if present completely escaping detection becomes remote. It is admitted that in some cases it was not possible to secure more than one batch of material of a given virus on a given host, but since this included four or five leaf portions taken from different leaves and often from different plants, it is maintained that in nearly all cases the examination of material was adequate for a diagnosis. Usually, however, two or more batches of material were secured at different times from different plants, and in some cases a considerable amount of material was worked through. For example, in the case of cucumber mosaic on tobacco, 16 leaf portions were examined from 10 different plants, and in the case of spot necrosis on the same host, 10 leaf portions from 8 plants. Comparing this with material of tobacco mosaic on tobacco, 41 leaf portions of the latter were examined from 34 different plants and in every case both striate material and x-bodies were found present. It is, therefore, believed that, where material was examined and no inclusions were observed, such inclusions were definitely not present and were not merely overlooked.

X-bodies and striate material were found to be associated with two other virus diseases in addition to ordinary tobacco mosaic, namely, yellow tobacco mosaic (fig. 1, E) and medium tobacco mosaic. In the former case, out of 9 hosts examined,[7] 4 contained inclusions of both types, 4 contained striate material only, and 1 showed no inclusions, while in the latter, out of 5 hosts, 1 showed both types of inclusion, 3 striate material only, and 1 no inclusions. (Tables 1, 2.) The inclusions were closely similar to those associated with ordinary tobacco mosaic in all cases except one. On petunia, yellow tobacco mosaic sometimes produced vacuolate bodies of a relatively enormous size, appearing more coarsely granular than the regular tobacco mosaic bodies, and considerably larger than the largest of these, though other smaller bodies were also observed in other cells of the same material. One of these large bodies is shown in Plate 1, D, *b*, closely associated with the host nucleus *n*, which appears slightly crushed. The presence of inclusions associated with these two diseases could be correlated to a certain extent with the intensity of symptoms produced. On the other hand, yellow tobacco mosaic, for example, produced a very striking mosaic pattern on tobacco leaves (fig. 1, E), although no x-bodies could be detected in the tissues.

It would, therefore, appear from a cytological standpoint that these two diseases were closely related to, though not identical with, ordinary tobacco mosaic. Although a study of differential symptoms indicates that they are not identical, the property studies conducted

[7] In addition to the host plants shown in Table 1, yellow tobacco mosaic was examined on *Solanum sisymbrifolium*, and striate material but no x-bodies were found.

by Johnson (*13*) also suggest a close relationship, since trials of the thermal death point, aging in vitro, and resistance to certain chemicals gave similar results for each virus. Since it has been shown that some viruses may be attenuated or modified under certain conditions (*12, 1*), and since yellow and medium tobacco mosaic were both originally obtained from tobacco plants growing in the field, it is conceivable that they may be naturally modified forms of the ordinary tobacco mosaic.

Generally speaking, cytological considerations are in favor of the view advanced by Johnson (*11*) that tobacco and other solanaceous plants may be affected by several different and distinct viruses.

A detailed investigation of the origin or development of the x-bodies in this group of plants was not attempted. However, by studying very young leaves, a few millimeters in length, taken from the tips of young tobacco plants affected with ordinary tobacco mosaic, it was possible to trace back the x-bodies to very small forms which appeared always in close contact with the host nucleus, irregular in shape, frequently crescent-shaped or somewhat triangular, staining similarly to the larger bodies, and appearing to consist of the same material, but without any vacuoles. Slightly larger bodies were also observed in which vacuoles were beginning to develop. These small bodies are evidently identical with certain forms described by Rawlins and Johnson (*29*) as appearing in the early stages of the disease and believed by these authors to be a possible young stage of the larger vacuolate forms. They also resemble early stages of the intracellular bodies present in mosaic corn plants as described by Kunkel (*15*).

VIRUS DISEASES OF THE POTATO

MATERIAL

The material for investigation of the potato virus diseases consisted of a number of different varieties of potato affected with one or more of the following diseases: Rugose mosaic, mild mosaic, supermild[8] mosaic, Montana crinkle, leaf-rolling mosaic, calico,[9] streak, leaf roll, crinkle mosaic, and tobacco mosaic. Tubers affected with each of the first eight diseases were obtained under these names from the department of botany and plant pathology, Oregon Agricultural Experiment Station; crinkle mosaic on Green Mountain potatoes was obtained from E. S. Schultz, United States Department of Agriculture; and rugose mosaic, on Bliss Triumph potatoes, was of Wisconsin origin. The tobacco mosaic virus was identical with that discussed in the previous section in connection with other solanaceous hosts and was secured on different varieties of potato by juice inoculation. These potato viruses were also transmitted to a limited extent to other varieties of potato by artificial inoculation. All plants except those inoculated with tobacco mosaic were grown in a cool greenhouse where the symptoms were usually distinct. Typical symptoms produced by three of the viruses studied are shown in Figure 3.

[8] The term "supermild" mosaic is used only tentatively in this paper.
[9] Possibly a virus disease.

METHODS

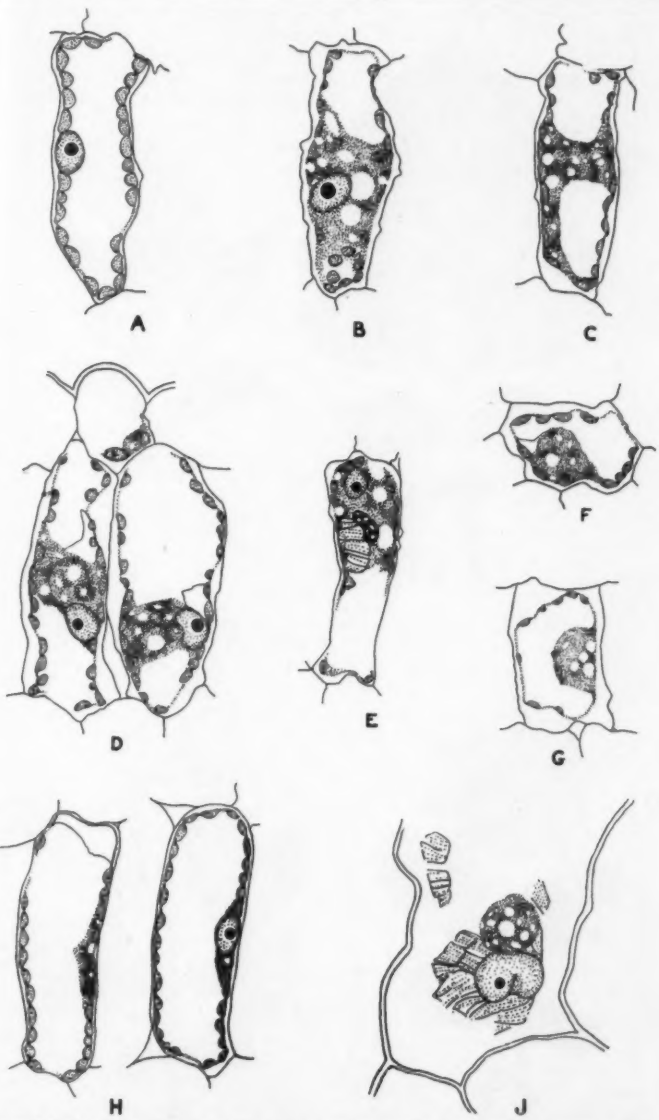
The material was examined by methods similar to those described in the previous section, both in the living and in the fixed condition. Formol acetic alcohol was employed throughout as fixing fluid, the fixed sections being stained with iron-alum haematoxylin as before.

RESULTS

The study of the potato virus diseases was complicated both by the inconsistencies in nomenclature of the different diseases, and by the difficulty in recognizing some of them on the basis of described symptoms, which constitute at present almost the only criterion. For example, "Montana crinkle," originally obtained on Rural New Yorker variety, when inoculated to Bliss Triumph potatoes, produced symptoms identical with those of rugose mosaic on this variety (fig. 3, D), and, according to Folsom (*6*), the name "crinkle," as sometimes used, is synonymous with rugose mosaic. On the other hand, the "crinkle mosaic" of Schultz and Folsom (fig. 3, C) is considered by these authors (*30*) as distinct from the "crinkle" of other systems of nomenclature. It was, therefore, considered impracticable to attempt a detailed study of the different virus diseases of this group upon different varieties of potato along the lines described in the previous section of this paper until some other more definite means of identification and classification of the viruses concerned has been established. However, since the occurrence of amoebalike, intracellular bodies has been reported by Smith (*32*) in association with mild mosaic [10] on Golden Wonder and President varieties of potato, it was believed that a study of such material as was already at hand would be of value; and, from cytological considerations, this group of diseases proved to be singularly interesting on account of their showing what are believed to be different stages in the formation of definite, vacuolate, protoplasmlike inclusions in the cells of the diseased tissues. These inclusions in their final stages appeared to be very similar to the bodies described by Smith. Smith, however, does not mention any other stages, which are, from the present standpoint, one of the most interesting features. Such bodies have been observed in association with so-called crinkle mosaic, rugose mosaic, Montana crinkle, leaf-rolling mosaic (fig. 3, B), mild mosaic, and supermild mosaic, and have so far been found in the chlorotic areas only of the leaves. They have been studied most fully in the case of crinkle mosaic on Green Mountain variety (fig. 3, C), and will be considered first in this connection.

In healthy Green Mountain potatoes, in all except the very young leaves, the cells of the palisade and mesophyll tissues were found to contain only a narrow layer of cytoplasm which lined the inner surface of the cell walls and in which were embedded the chloroplasts and usually the nucleus. (Pl. 3, F, and fig. 2, A.) In the palisade cells, the nucleus was occasionally suspended in a narrow bridge of cytoplasm extending across the cell near the center. The cells of the epidermis and vein parenchyma contained a similar cytoplasmic layer and nucleus, but very few or no chloroplasts. In the fixed material, the cytoplasm appeared frequently somewhat shrunken or perhaps

[10] The type of mosaic is not mentioned in his publication, but in correspondence Smith states that it was "what might be called mild mosaic."



IG. 2.—Cell inclusions in the leaves of potato in association with certain virus diseases. × 700. A, Healthy Green Mountain potato. Palisade cell. B, Crinkle mosaic on Green Mountain potato. Indefinite mass of vacuolate material in palisade cell. C, Crinkle mosaic on Green Mountain potato. Vacuolate material as a bridge across palisade cell. D, Crinkle mosaic on Green Mountain potato. Vacuolate material in palisade cells and epidermal cell. Brown material in palisade cells shown in outline. E, Tobacco mosaic on Rural New Yorker potato. X-body, striate material, and vacuolate material in palisade cell. F, Crinkle mosaic on Green Mountain potato. Partially rounded mass of vacuolate material in mesophyll cell. G, Crinkle mosaic on Green Mountain potato. Body attached to host cytoplasm in parenchymatous cell adjacent to large vein. H, Rugose mosaic on Bliss Triumph potato. Small amount of vacuolate material adjacent to wall of palisade cell. Brown material shown in outline at top of left-hand cell. J, Tobacco mosaic on Rural New Yorker potato. X-body, striate material, and host nucleus in hair cell.

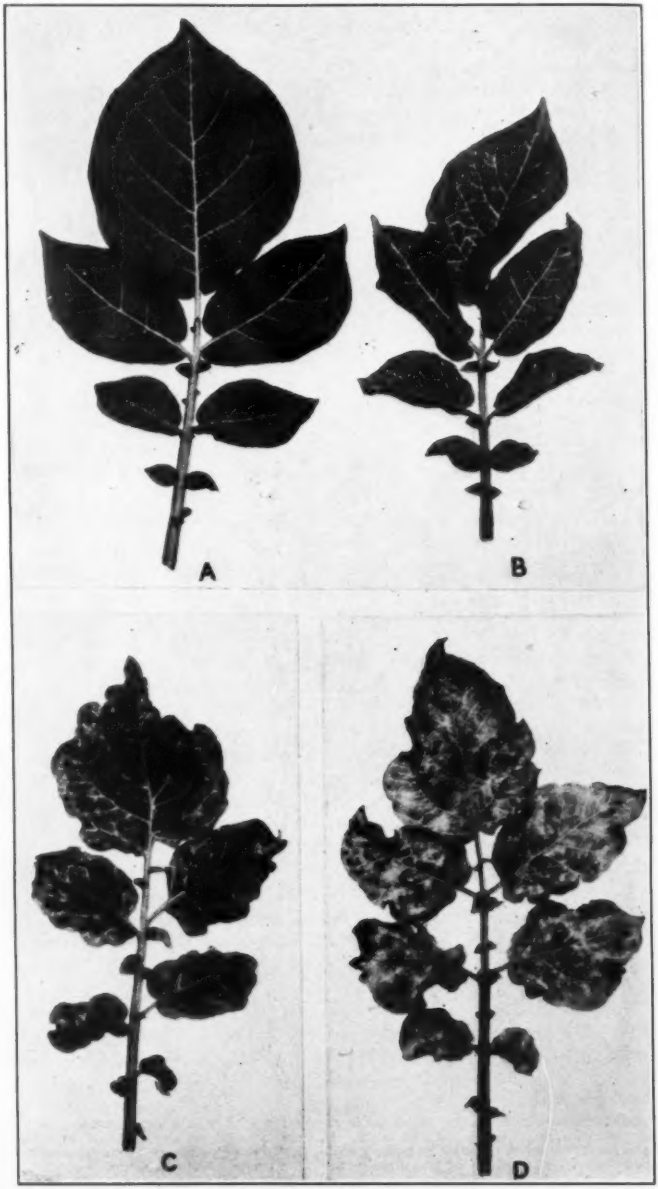


FIG. 3.—Types of leaf symptoms produced on potato by different virus diseases: A, Healthy Green Mountain potato; B, leaf-rolling mosaic on Green Mountain potato; C, crinkle mosaic on Green Mountain potato; D, rugose mosaic on Bliss Triumph potato

partially dissolved, and showed only as a very narrow layer. In plants affected with crinkle mosaic, on the other hand, in leaves on which the symptoms of mottling had become pronounced, many of the cells of the chlorotic areas were found to contain an excessive amount of fairly dense, vacuolate, protoplasmlike material, which was especially noticeable in the palisade and mesophyll cells. This material stained a blue-gray to deep blue-black shade with the iron-alum haematoxylin, and was present in varying amounts in different cells of the same tissue. In the palisade cells it was frequently seen as a bridge extending across the cell (pl. 3, A, p_3, C, p_1, and fig. 2, C, D), as a dark band or mass lying against one or more of the walls (pl. 3, A, p_1, p_2, C, p_2), or as an irregular, more diffuse mass, occupying a large portion of the cell (pl. 3, D, p, and fig. 2, B). In the mesophyll tissue it took the form of an irregular mass (pl. 3, B, p_2) or a narrower band lying against the cell wall (pl. 3, B, p_1). Similar material occurred in the epidermal cells and in the parenchymatous tissue surrounding the larger veins. In other cells of the same tissues the material appeared as if partially rounded up and approaching in form that of a definite "body." (Pl. 3, C, p_4 and fig. 2, F.) In still other cells the same material was present but in the form of a quite definite, rounded mass or "body" (pl. 3, D, b_1, b_2) evidently similar to the "amoeba-like bodies" described by Smith. The dark green areas of the leaf, on the other hand, contained little or none of this vacuolate material. Portions of leaf material taken a week or more later from the same plant and from leaflets of about the same size were found to contain definite bodies of this type in the chorotic areas in much greater abundance, while the irregular or indefinite forms of the same vacuolate substance were more rare. Such bodies are shown in Plate 1, E and F, and in Plate 2, A and B. The chloroplasts in the chlorotic areas were now found to be gorged with starch in both palisade and mesophyll tissues. The bodies were usually round or oval in shape, sometimes rather irregular in outline, but very definite. They were about twice as large in diameter as the host nucleus, and appeared to consist of a coarsely granular material containing one or more vacuoles. They were often situated in close contact with the nucleus, sometimes appearing attached to it, but at other times lying free. They seldom showed any clear indication of a limiting membrane, nor was any structure observed which could be interpreted as a nucleus, either in them or in the irregular vacuolate masses previously described. The bodies were somewhat similar to those of tobacco mosaic, but were on an average considerably larger; they usually contained fewer and larger vacuoles and were of a more coarsely granular nature. Occasionally, forms were observed which appeared to be drawn out at one point into a narrow projection resembling an appendage. The bodies often appeared to be attached to, or in close union with, the cytoplasmic layer of the host cell. (Fig. 2, G.) Material taken from leaves very soon after these had developed symptoms contained no definite bodies, though a certain amount of vacuolate material was present in irregular or indefinite forms. Since the definite bodies apparently do not occur in the tissues until somewhat later in the development of the disease, and since a series of intermediate forms can be found between the irregular masses of vacuolate material and the bodies, it is suggested that the

latter are formed by a process of rounding up of the vacuolate material in the host cells.

The bodies and other forms of vacuolate material were studied also in fresh, unstained tissues by means of free-hand razor sections mounted in water or in physiological salt solution. In this condition, the vacuolate material appeared similar in form and structure to that observed in the stained preparations. It was seen to consist of a colorless, hyaline substance, protoplasmic in appearance and closely resembling the cytoplasm of the cell, with which it was in apparent continuity. In the stained slides it was observed that, wherever plasmolysis had occurred in the process of fixation, the vacuolate material had drawn away from the cell wall together with the cytoplasmic layer (pl. 3, C, p_4), indicating some kind of union with, or attachment to, the cytoplasm, and suggesting a common origin.

As to the nature of this material, it is not believed to be a degeneration product of the nucleus, as Smith suggested, since many of the cells which contain the substance, either as a definite body or as an indefinite mass, contain also an apparently normal nucleus. (Pl. 2, A, pl. 3, A, B, and fig. 2, B, D.) It is suggested, however, that it may be cytoplasmic in nature, representing some modification or abnormal form of the host cytoplasm.

Bodies and vacuolate material similar to those of crinkle mosaic have been observed also in association with rugose mosaic on Bliss Triumph, Green Mountain, and Early Ohio potatoes, with "Montana crinkle" on Rural New Yorker potatoes, with leaf-rolling mosaic on Green Mountain potatoes and with mild and supermild mosaics on American Wonder potatoes. Plate 2, C, shows a body in the leaf of Green Mountain potato affected with rugose mosaic, and Plate 2, D, a mesophyll cell of the same tissue completely filled with the vacuolate material. Plate 2, E, shows a large mass of vacuolate material in a leaf-hair of American Wonder potato affected with supermild mosaic. With the exception of "Montana crinkle" on Rural New Yorker variety, however, the bodies were of less frequent occurrence in all these cases, though in Bliss Triumph potatoes affected with rugose mosaic the vacuolate material was extremely abundant, occurring in almost every cell. (Pl. 3, E.) Here the nuclei frequently appeared at first sight as if partially dissolved or degenerating (pl. 3, E, n), but a more careful examination showed that this was due to the partial concealment of the nuclei by the vacuolate material, and in cells containing definite bodies the nuclei were normal in appearance. In earlier stages of the development of this disease on Bliss Triumph variety, the vacuolate material was much less abundant and was apparently limited to narrow bands or masses lying against the wall of the cell, and containing few or no vacuoles. (Fig. 2, H.) In American Wonder variety affected with mild and supermild mosaics, the vacuolate material was not very abundant in the cells and definite bodies were of relatively rare occurrence. This may, perhaps, be correlated with the comparatively mild symptoms produced by these two mosaics. Similar vacuolate material, but no bodies, were observed also in association with supermild mosaic on Burbank, and with rugose mosaic on Cobbler, King, and Burbank varieties, and it is possible that, in some cases at least, definite bodies would have formed had the plants been kept for a sufficient length of time. On the other hand, no such vacuolate material was observed in association with

calico and leaf roll on Burbank or with streak on Bliss Triumph variety.

Further investigations of the origin and development of this vacuolate material are in progress.

A second type of inclusion was present in the stained material in association with all the virus diseases studied with the exception of calico, leaf roll, and streak. This consisted of a brownish substance occurring in irregular masses in the different tissues of the leaf, sometimes in cells which contained the vacuolate material, and sometimes in cells where this was not present. (Pl. 2, A, D, *m*, and fig. 2, D, H.) This substance was not observed in this form, however, in fresh, unstained tissues and its origin has not yet been determined. No striate material was found associated with any of these diseases of the potato.

On inoculation of the ordinary tobacco mosaic virus to potato (Rural New Yorker and Green Mountain varieties) both x-bodies and striate material appeared in abundance in the cells and were similar to those occurring in other host plants affected with this virus. (Pl. 2, F, and fig. 2, J.) In addition to these inclusions, many cells were found to contain irregular masses of vacuolate material similar to that associated with the potato mosaic diseases already described. This material stained somewhat more lightly than the tobacco bodies and was readily distinguishable from the latter on this account as well as by its irregular form and larger vacuoles. Frequently a single cell was found to contain a tobacco x-body and striate material as well as the vacuolate material. (Fig. 2, E.)

In the potato virus group, cytological evidence at present suggests that some, at least, of the viruses may be closely related, since vacuolate, protoplasmlike bodies of a similar nature are associated with several of these diseases. On the other hand, since no inclusions were found present in the case of leaf roll, calico, and streak, their production is evidently characteristic only of certain virus diseases of potato.

DISCUSSION

The results described in the preceding pages have a bearing both on the proposed classification of plant viruses (*13*) and on the nature of the x-bodies, or protoplasmic inclusions, present in certain mosaic-diseased tissues.

In the first place, it has not been found possible, as was originally hoped, to establish, to any great extent, any means of classification of viruses on the basis of microscopical features of the diseased tissues. It has, however, been shown that the production of cell inclusions in the tissues of various host plants is characteristic of certain viruses only, and must, therefore, be ascribed to some specific character or property of these viruses and not of the host plants. It follows that the presence of such inclusions may in these cases be employed as an additional means of identification of the viruses in question, and it is believed that in the future such features will become of more general application in the recognition of certain virus diseases in cases where a single host plant is susceptible to more than one virus. The need of some system of classification of plant viruses is already apparent and has been discussed in detail elsewhere (*13*), and it is believed that cytological features such as those now under consideration will prove

of value in supplementing other known diagnostic characters in any established system of classification.

The more fundamental considerations, however, are those which relate to the nature of the x-bodies or protoplasmic inclusions, which have been variously interpreted as protozoa or other living organisms and the causal agent of the disease in question, and as certain degeneration or reaction products of the host cells.

The constant association of these bodies with tobacco mosaic on various host plants is in harmony with the causal organism theory. It must, however, be remembered that the striate material is also a constant feature of the disease, yet this substance is crystalline in nature and can hardly be a form of living organism. On the other hand, if the x-bodies are of a causal nature, it is to be expected that they would be constantly associated with a large number of, if not all, mosaic diseases, Up to the present, they have been reported in connection with 10 different plant diseases, namely, mosaic of tobacco, corn, sugar cane, Fiji disease of sugar cane, mosaic of wheat, *Hippeastrum equestre*, *H. johnsonii*, Chinese cabbage, potato, and dahlia. Few reports have yet been made of plant virus diseases with which such bodies are definitely not associated. McKinney (*21*) was unable to find definite inclusion bodies in the case of mosaic of cucumber, raspberry, or sweet clover, though he does not consider these negative results as final. Smith (*31*) recently states that intracellular bodies were not observed in mosaic Aquilegia and only rarely in pokeweed, though she gives no indication of the type of mosaic with which she was working. Evidence is presented in this paper of eight virus diseases of solanaceous plants with which such inclusions definitely do not occur, at least under greenhouse conditions. These are: Cucumber mosaic, speckled tobacco mosaic, mild tobacco mosaic, spot necrosis, ring spot, bleaching mosaic, tomato stem necrosis, and petunia mosaic. Neither have inclusions been observed so far in association with leaf roll, streak, or calico of potato. The mosaic of petunia described by Smith (*31*) which produced inclusion bodies and striate material similar to those of tobacco mosaic on tobacco is believed to be ordinary tobacco mosaic on petunia, and not the petunia mosaic as described by Johnson (*11*) and now under consideration. It is believed furthermore, that a number of other mosaic diseases will be found to show no inclusion bodies in the host tissues, and it is hoped that, in the future, investigators who look for such bodies will make a definite report even in the case of negative results. It is, of course, possible that all virus diseases are not due to a similar cause, and this appears probable when one considers diseases such as the "yellows" group on the one hand, and the "mosaic" group on the other. The diseases under discussion, however, for the most part come within the "mosaic" group, and it is more difficult to conceive of such diseases as tobacco and cucumber mosaic, for instance, as falling into different categories since these show so many striking resemblances.

While it is too early yet to draw final conclusions concerning the vacuolate bodies associated with the potato virus diseases, the evidence bearing on their origin is very suggestive. If these bodies actually represent some modification or abnormal form of the host cytoplasm, then it is likely that the x-bodies of tobacco and other mosaics are also products of diseased cells and not the causal agents of the diseases with which they are associated.

SUMMARY

Two types of cell inclusion, namely, x-bodies and striate material, previously described in association with tobacco mosaic on tobacco, are found to be a constant feature of this disease on solanaceous host plants which develop definite symptoms of mottling or chlorosis.

Similar inclusions are associated with two other virus diseases of solanaceous plants, namely, yellow tobacco mosaic and medium tobacco mosaic, which appear from various considerations to be closely related to ordinary tobacco mosaic.

Eight other virus diseases are found to produce no inclusions of either type in any of the solanaceous hosts examined.

It is believed that the presence or absence of such inclusions will prove of more general application in the identification of certain different virus diseases in cases where a single host species is susceptible to more than one virus.

An abnormal, vacuolate, protoplasmlike material occurs in the cells of potato in association with several different virus diseases. This material appears in various forms, either as definite bodies or as irregular masses whose appearance suggests intermediate stages between the bodies and the host cytoplasm.

It is believed that the evidence presented in this paper is on the whole in favor of the view that the x-bodies are not of the nature of a causal organism.

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